Carbohydrate Polymers 117 (2015) 468–475

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation, characterization, and antimicrobial activity of chitin nanofibrils reinforced carrageenan nanocomposite films

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ARTICLE INFO

Article history:
Received 27 July 2014
Received in revised form 7 October 2014
Accepted 7 October 2014
Available online 17 October 2014

Keywords:
Chitin
Nanofibrils
Carrageenan
Nanocomposite films
Antimicrobial activity

ABSTRACT

Present study illustrates the preparation of chitin nanofibrils (CNF) reinforced carrageenan nanocomposite films by the solution-casting technique. CNF was prepared by acid hydrolysis of chitin, followed by high speed homogenization and sonication. FTIR result demonstrated that the chemical structure of chitin had not changed after acid hydrolysis. However, the crystalinity of CNF was found to be higher than chitin. The crystallite size of chitin and CNF was 4.73 and 6.27 nm, respectively. The char content at 600 °C of chitin (19.2%) was lower than the CNF (25%). The carrageenan/CNF composite films were smooth and flexible and the CNF was dispersed uniformly in the carrageenan polymer matrix. The tensile strength and modulus of carrageenan film were increased significantly ($p < 0.05$) after CNF reinforcement with up to 5 wt%, however, elongation at break, water vapor permeability, and transparency decreased slightly. Carrageenan/CNF nanocomposite films showed strong antibacterial activity against a Gram-positive food-borne pathogen, *Listeria monocytogenes*.

1. Introduction

Concerns on the environment and exhaust of natural resources caused by the non-biodegradable petroleum-based plastics have attracted attention on the development of environmentally benign polymers and polymer nanocomposites for the applications in food packaging and other value added utilities. Renewable and abundantly available biopolymers are the most viable alternative for the production of green materials in the near future. Nature has provided various natural biopolymers such as polysaccharides (cellulose, starch, and chitosan) and proteins (soy protein, wheat protein, casein, and gelatin). Utilization of natural biopolymers for making biodegradable packaging films, edible film, and coating materials have been increased considerably in the past decades (Rhim, Park, & Ha, 2013). Among such natural biopolymers, carrageenan is one of the promising biopolymers since it is abundantly obtained from renewable resources such as red edible seaweeds, which has strong gel forming ability, biocompatibility, and is environmentally friendly. Carrageenan is a linear sulfated polysaccharide extracted from various species of the *Rhodophyta* (marine red algae) (Necas & Bartosikova, 2013). Carrageenan has been frequently used to prepare biodegradable or edible packaging films. However, poor performances such as lower water vapor barrier and relatively lower mechanical properties are the main limitations of such biopolymer-based films. To overcome these problems, a number of research works have been performed by reinforcing nanofiller materials (Martins et al., 2013; Rhim, Park, & Ha, 2013). Inorganic (metallic nanoparticles, and nanoclays) or organic (cellulose nanowhiskers, starch nanoparticles, and chitosan nanoparticles) nanofillers have been used as reinforcing materials. Reinforcement of organic nanofillers is preferred over inorganic nanofillers because of their completely biodegradable nature.

Among all organic nanofillers, chitin nanofibrils (CNF) is one of the most promising nanofiller. Chitin is a linear polysaccharide, made up of β-(1-4)-linked 2-acetamido-2-deoxy-D-glucopyranose units which may be deacetylated to some extent (Muzzarelli, 2011a). Chitin is the second most abundant biomaterial after cellulose in the world. Chitin can be obtained from the cell wall of fungi, the exoskeleton of arthropods such as crustaceans (e.g., crabs, lobsters, and shrimps) and insects, the radula of molluscs, and the beaks and the internal shells of cephalopods including squids and octopuses (Muzzarelli, 2011a, Muzzarelli et al., 2012)Chitin upon acid hydrolysis can form crystalline nanofibrils and nanowhiskers, which have been recently explored in nanotechnology applications (Mincea, Negrulescu, & Ostafe, 2012; Muzzarelli, 2011b). Marine chitin lends itself to the isolation of nanofibrils, otherwise

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http://dx.doi.org/10.1016/j.carbpol.2014.10.010
0144-8617/

called nanocrystals or whiskers; this matter has been the subject of various review articles (Mincea et al., 2012; Muzzarelli, 2011a; Muzzarelli, 2011b; Muzzarelli, 2012; Muzzarelli et al., 2012). Reports are available on the formations of chitin whiskers using acid hydrolysis (Ifuku et al., 2009; Revol & Marchessault, 1993), however, few studies have been reported on the preparation of biopolymer-based films reinforced with chitin nanofibrils. To the best of our knowledge, reports on the reinforcement of carrageenan-based biopolymer films with chitin nanofibrils (CNF) have not been available in the literature.

The main objective of the present study was to synthesize CNF from chitin and preparation of carrageenan/CNF nanocomposite films. The CNF was characterized by various analytical techniques. The effects of CNF concentrations on the optical, mechanical, and water vapor barrier properties of carrageenan/CNF nanocomposite films were studied.

2. Materials and methods

2.1. Materials

Food grade carrageenan (HGE-A, pure κ-carrageenan, viscosity [1.5% aq. solution at 75 °C] 60 cps) was obtained from MSC Co. Ltd. (Sungnam city, Kyunggido, Korea). Glycerol and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (St Louis, MO, USA). Chitin (viscosity [0.5%-AA & CS at 20 °C]: 800–940 cps, ash < 5%, particle size: 3 mm) obtained from crab shell was procured from YB Bio Co., Ltd. (Youngduk, Kyungbuk, Korea) and used without any modification. Food-borne pathogenic bacteria, *Escherichia coli* O157:H7 ATCC 43895 and *Listeria monocytogenes* ATCC 15313, were obtained from Korean Collection for Type Cultures (KCTC, Seoul, Korea). The bacterial strains were cultured in tryptic soy broth (TSA) and brain heart infusion broth (BHI) agar media and subsequently stored at 4 °C for further analysis.

2.2. Isolation of chitin nanofibrils

Chitin nanofibrils (CNF) were isolated from the chitin by acid hydrolysis. For this, 5 g of chitin was hydrolyzed with 3 N HCl (chitin to acid ratio of 1:20) by refluxing for 3 h at 60 °C under strong agitation. The reaction was quenched by adding an excess of distilled water to the reaction mixture and the resulting mixture was cooled to the room temperature. The unhydrolyzed fibers were filtered and the filtrate was centrifuged at 4000 rpm for 20 min using a bench-top centrifuge (Hanil Scientific Centrifuge, Incheon, Kyonggido, Korea). The precipitate obtained was repeatedly washed with distilled water and the supernatant was discarded until it became turbid. During this process, the pH of the suspension was above 5. Thereafter, the suspension was homogenized using a high speed homogenizer (T25 basic, Ika Labotechnik, Janke & Kunkel Gmbh & Co., KG Staufen, Germany) at 7000 rpm for 30 min followed by sonication for 5 min in an ice bath using a high intensity ultrasonic processor (Model VCX 750, Sonics & Materials Inc., Newtown, CT, USA). Finally, the suspension was subjected to dialysis against distilled water until neutral pH was attained. The suspension was stored in a refrigerator at 4 °C for further analysis.

2.3. Preparation of films

Carrageenan and carrageenan/CNF nanocomposite films with different concentration of CNF (3, 5, and 10 wt% based on carrageenan) were prepared using a solution casting method (Rhim, 2011). For this, predetermined amount of CNF was dispersed in 150 mL of distilled water and stirred for 1 h using a magnetic stirrer. The fully wetted suspensions were homogenized using a high shear mixer (T25 basic, Ika Labotechnik, Janke & Kunkel Gmbh & Co., KG Staufen, Germany). Thereafter, 1.2 g of glycerol was mixed in the suspension as a plasticizer and mixed vigorously for 20 min on a magnetic stirrer. Then, 3 g of carrageenan was added slowly in the above solution and mixed vigorously for 30 min at 95 °C using a magnetic stirrer and cast evenly onto a leveled Teflon film (Cole-Parmer Instrument Co., Chicago, IL, USA) coated glass plate ($24 \times 30\ cm^2$). The films were allowed to dry for about 24 h at room temperature. The dried films were peeled off from the casting plates and conditioned in a constant temperature humidity chamber set at 25 °C and 50% relative humidity (RH) for at least 48 h before further analysis. The control film of carrageenan (without CNF) was also prepared following the same method described above.

2.4. Characterization

Particle size of CNF was analyzed using a particle size analyzer (Brookenhaven Instruments Corp., New York, USA). 20 μL of homogeneous CNF suspension was diluted into 3 mL of deionized distilled water and subjected to the particle size analyzer.

Fourier transform infrared (FT-IR) spectra of chitin and CNF were obtained using an attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectrophotometer (TENSOR 37 Spectrophotometer with OPUS 6.0 software, Billerica, MA, USA) in the range of $4000–500\ cm^{-1}$.

X-ray diffraction (XRD) pattern of chitin, CNF and carrageenan/CNF nanocomposite films was analyzed using a PANalytical Xpert pro MRD diffractometer (Amsterdam, Netherlands). The XRD spectra were recorded using Cu-Kα radiation (wavelength of 0.1546 nm) and a nickel monochromator filtering wave at a voltage and current of 40 kV and 30 mA, respectively. The diffraction patterns were obtained at diffraction angles between 5 and 50° with a scanning rate of 0.4°/min at room temperature. The degree of crystallinity (DC) of chitin and CNF was calculated using following equation (El-Nesr, Raafat, Nasef, Soliman, & Hegazy, 2013):

$$DC = (I_{110} - I_{am})/I_{110} \quad (1)$$

where, I_{110} is the intensity of the peak at (110) lattice (at $2\theta = 19.1°$) and I_{am} is the intensity of the peak at $2\theta = 16°$ (amorphous region). And the crystallite size (D) of the fiber was calculated by using the Scherrer equation (Das et al., 2009).

$$D = K\lambda/\beta_{1/2}\cos\theta \quad (2)$$

where, K is a constant (0.94), λ is the *X*-ray wavelength ($\lambda = 0.154056$ nm), $\beta_{1/2}$ is the full width at the half maximum of the deflection peak (FWHM), and θ is Bragg's angle.

The microstructure of chitin and CNF and the surface morphology of carrageenan/CNF nanocomposite films were observed using a field emission scanning electron microscopy (FE-SEM, S-4800, Hitachi Co., Ltd., Matsuda, Japan) operated with an acceleration voltage of 10 kV and current of 10 μA after coating the samples with platinum (Pt) using a vacuum sputter coater.

2.5. Thermal stability

Thermal stability of chitin and CNF was determined by thermogravimetric analyzer (Hi-Res TGA 2950, TA Instrument, New Castle, DE, USA). For this, about 5 mg of sample was taken in a standard aluminum cup and heated from 30 to 600 °C at heating rate of 10 °C/min under a nitrogen flow of $50\ cm^3$/min. An empty cup was used as a reference.

2.6. Color and transparency

The surface color of the films was measured using a Chroma meter (Konica Minolta, CR-400, Tokyo, Japan). A white standard