



Characterization of bionanocomposite films prepared with agar and paper-mulberry pulp nanocellulose

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ABSTRACT

Crystallized nanocellulose (CNC) was separated from paper-mulberry (*Broussonetia kazinoki* Siebold) bast pulp by sulfuric acid hydrolysis method and they were blended with agar to prepare bionanocomposite films. The effect of CNC content (1, 3, 5 and 10 wt% based on agar) on the mechanical, water vapor permeability (WVP), and thermal properties of the nanocomposites were studied. Changes of the cellulose fibers in structure, morphology, crystallinity, and thermal properties of the films were evaluated using FT-IR, TEM, SEM, XRD, and TGA analysis methods. The CNC was composed of fibrous and spherical or elliptic granules of nano-cellulose with sizes of 50–60 nm. Properties of agar film such as mechanical and water vapor barrier properties were improved significantly ($p < 0.05$) by blending with the CNC. The tensile modulus and tensile strength of agar film increased by 40% and 25%, respectively, in the composite film with 5 wt% of CNC, and the WVP of agar film decreased by 25% after formation of nanocomposite with 3 wt% of CNC. The CNC obtained from the paper-mulberry bast pulp can be used as a reinforcing agent for the preparation of bio-nanocomposites, and they have a high potential for the development of completely biodegradable food packaging materials.

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1. Introduction

Not only concerns on the exhaust of natural resources and soaring oil price, but also environmental awareness caused by non-biodegradable plastic packaging materials have attracted considerable attention on the development of entirely biodegradable packaging materials from renewable and sustainable resources (Abdul Khalil, Bhat, & Ireana Yusra, 2012; Khan, Huq, Khan, Riedl, & Lacroix, 2014; Reddy, Vivekanandhan, Misra, Bhatia, & Mohanty, 2013; Sorrentino, Gorras, & Vittoria, 2007). Biodegradable packaging materials are usually produced from renewable, biological raw materials such as starch, cellulose, and other biopolymers (Tang, Kumar, Alavi, & Sandeep, 2012). Among them, cellulose is the most abundant natural biopolymers in the world and it is increasingly being used for the preparation of composites with other biopolymers (Faruk, Bledzki, Fink, & Sain, 2012; Jawaid & Abdul Khalil, 2011; John & Thomas, 2008; Khan et al., 2014; Siqueira, Bras, & Dufresne, 2010). Cellulose is a semicrystalline linear polysaccharide composed of β -1,4 linked glucopyranose units, with polymer chains associated by hydrogen bonds forming

bundles of fibrils, which consist of highly ordered crystalline domains and disordered amorphous domains. The crystalline domains can be isolated in nanoscale with highly ordered and regular rod-like nanocrystals, after removing the amorphous domains by acid hydrolysis, which is called cellulose nanofiber, crystalline nanocellulose, or nanowhisker (De Souza Lima & Borsali, 2004). The production of cellulose nanofibers and their application in nanocomposite materials has gained increasing attention due to the profound improvement of properties of the composites such as increased mechanical and decreased gas barrier properties (Lavoine, Desloges, Dufresne, & Bras, 2012; Siró & Plackett, 2010). Such benefits of nanocellulose as nanofiller are mainly caused by their high strength and stiffness combined with low weight, as well as their biodegradability, biocompatibility, and renewability (Peng, Dhar, Liu, & Tam, 2011). Compared with micro-sized cellulose, cellulose nanofibers are more effective to reinforce polymers due to interactions between the nanosized elements that form a percolated network connected by hydrogen bonds when the nanofibers are well dispersed in the polymer matrix (Khan et al., 2014). In addition, nanocellulose can be obtained from cheap and abundant renewable natural resources such as wood, plants, vegetables, and other agricultural residues and agricultural processing wastes (Klemm et al., 2011). The properties of nanocellulose are known to be strongly dependent not only on the isolation method but also on

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the source of the nanofibers (Azizi Samir, Alloin, & Dufresne, 2005). Nanocelluloses have been separated from various source of plant materials and demonstrated that they were good fillers to improve the mechanical properties of biopolymer films such as starch (Da Silva, Pereira, & Druzian, 2012), alginate (Huq et al., 2012), and chitosan (Khan et al., 2012) films.

As one of the renewable and sustainable resources of cellulose fibers, mulberry bast fiber has attracted as an environmentally-friendly new fiber source (Li et al., 2009; Lim & Choi, 2008; Qu & Wang, 2011; Walia, 2013). Mulberry (*Broussonetia kazinoki* Siebold) trees are extensively grown in Asian countries like China and India for feeding silk worms with their leaves. Thousands tons of mulberry branches consisting of bast and stalk are produced for firewood or agro-wastes every year. The bast of the mulberry tree has been used for paper manufacturing since the mulberry fibers are very long (6–20 mm; average 10 mm) and provide great strength to the paper (Lim & Choi, 2008; Walia, 2013). The chemical composition and the structural characterization of the mulberry pulp fibers and the chemically treated fibers were reported (Qu & Wang, 2011). The extraction and characterization of nanowhiskers from mulberry bark fibers were also reported in the literature (Li et al., 2009). However, few studies on the isolation of nanocellulose from mulberry pulp and its application in the preparation of nanocomposites with biopolymers have been appeared in the literature.

Among the natural biopolymers, agar has a potential for making biodegradable films for packaging applications (Rhim & Wang, 2013). Agar is a polysaccharide derived from red algae. Agar contains two components such as agarose and agarpectin. Agar is soluble in hot water and it forms biodegradable film when added with proper plasticizer. Agar has been also successfully used to make nanocomposites with nanoclay and reported that the mechanical and water vapor permeability have been improved with the addition nanoclay (Rhim, 2011; Rhim, Lee, & Hong, 2011). However, no reports on crystallized nanocellulose reinforced agar-based composites are available in the literature.

The main objective of the present study was therefore to prepare agar-based bionanocomposite films reinforced with crystallized nanocellulose isolated from paper mulberry bast pulp. For this, crystallized nanocelluloses were isolated from mulberry pulp fibers and characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and thermogravimetric analysis (TGA). In addition, the agar-based nanocomposite films with nanocellulose were prepared by the solution casting method and their film properties were evaluated.

2. Materials and methods

2.1. Materials

Food grade agar was obtained from Fine Agar Co., Ltd. (Damyang, Jeonnam, Korea). Glycerol, sulfuric acid and sodium hydroxide were obtained from Sigma Aldrich (St Louis, MO, USA). Bleached pulp of paper mulberry bast with a cellulose content of 85% was obtained from Chunyang Paper Co., Ltd. (Jeonju, Korea).

2.2. Isolation of nanocellulose

For the pretreatment, the mulberry pulp was immersed in water after cut into small pieces (5 cm long) and the cellulose fibers were separated using a laboratory scale valley beater for 60 min. The separated fibers were dried in a drying oven at 105 °C for 24 h.

The mulberry cellulose fibers were subjected to acid hydrolysis for the isolation of nanocellulose. For this, 5 g of mulberry

cellulose fiber was hydrolyzed with sulfuric acid (47% H₂SO₄ with fiber to solution 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 then cooled to the room temperature. Then, the suspension was centrifuged at 4000 rpm for 20 min using a bench-top centrifuge (Hanil Scientific Centrifuge, Incheon, Kyonggido, Korea) repeatedly and the supernatant was discarded until it became turbid. Subsequently, the fractions were washed with distilled water by centrifugation. Under this condition, the pH of the suspension was above 5. The suspension was then sonicated using a high intensity ultrasonic processor (Model VCX 750, Sonics & Materials Inc., Newtown, CT, USA) for 5 min in an ice bath. Then the suspension was subjected to dialysis with water until neutrality was attained. After this, the suspension was freeze-dried to get crystalline nanocellulose (CNC) of the mulberry pulp fiber.

2.3. Characterization

FT-IR spectra of fiber samples 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 the CNC was analyzed using a PANalytical Xpert pro MRD diffractometer (Amsterdam, Netherlands). The XRD spectra were recorded using Cu-K α 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 mulberry fiber and its CNC was calculated using following equation (Gumuskaya, Usta, & Kirci, 2003):

$$DC = (I_{002} - I_{am})/I_{002} \quad (1)$$

where I_{002} is the intensity of the 002 peak (at $2\theta = 22^\circ$) and I_{am} is the intensity of the peak at $2\theta = 16^\circ$. 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 ($\lambda = 0.1546$ 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 mulberry fiber and the surface morphology of bionanocomposite 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 μ A after coating the samples with osmium (Os) using a vacuum sputter coater.

For the TEM observation, the CNC was dispersed in distilled water (0.01 wt%) and about 8 μ L of the CNC solution was deposited onto a glow-discharged carbon-coated TEM grid (Farmvar film on 200 square mesh copper grid, Ted Pella Inc., Redding, CA, USA). After 10 min, the excess liquid was removed by blotting with a filter paper and allowed to dry under the ambient condition. The microstructure of CNC was observed using a transmission electron microscopy (TEM, JEM-2100F, JEOL Ltd., Tokyo, Japan). The length and diameter of CNC were determined using the internal scale of the TEM images.

2.4. Preparation of films

Agar and agar-based nanocomposite films were prepared using a solution casting method following the method of Rhim et al. (2011). Agar film was prepared by dissolving 4 g of agar powder

in 150 mL of distilled water with 2 g of glycerol as plasticizer while mixing 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 cm × 30 cm), then dried for about 24 h at room temperature. In addition, agar/CNC nanocomposite films with different concentration of nanocellulose (1, 3, 5, and 10 wt% based on agar weight) were prepared. For this, pre-determined amount of CNC 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 Labortechnik, Janke & Kunkel GmbH & Co., KG Staufen, Germany) at 12,000 rpm for 10 min. Then, 4 g of agar and 2 g of glycerol were dissolved into the CNC suspension while mixing vigorously for about 30 min at 95 °C using a hot plate and then cast evenly onto a leveled Teflon film coated glass plate and followed the same procedures as described above. The dried films were peeled off from the casting plate and conditioned in a constant temperature humidity chamber set at 25 °C and 50% relative humidity (RH) for at least 48 h before further test.

2.5. Color and transparency

Surface color of the films was measured using a Chroma meter (Konica Minolta, CR-400, Tokyo, Japan). A white standard color plate ($L = 97.75$, $a = -0.49$ and $b = 1.96$) was used as a background for color measurements. Hunter color (L , a , and b) values were averaged from five readings from each sample. The total color difference (ΔE) was calculated as follows:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3)$$

where ΔL , Δa , and Δb are difference between each color values of standard color plate and film specimen, respectively.

The percent transmittance at wavelength of 660 nm (T_{660}) was determined using a UV-vis spectrophotometer (Model 8451A, Hewlett-Packard Co., Santa Alara, CA, USA) (Rhim, 2011).

2.6. Tensile properties

Tensile properties such as tensile strength (TS), elongation at break (E), and elastic modulus (EM) of each film samples were evaluated with a Model 5565 Instron Universal Testing Machine (Instron Engineering Corporation, Canton, MA, USA) in accordance with the ASTM Method D 882-88. For this, films were cut into 2.54 cm × 15 cm strips using a precision double blade cutter (Model LB.02/A, Metrotech, S.A., San Sebastian, Spain). The machine was operated in tensile mode with an initial grip separation and cross-head speed set at 50 mm/min and 50 mm/min, respectively (Rhim, 2011). The TS (Pa) was determined by dividing the maximum load (N) by the initial cross-sectional area (m²) of the film sample, and the E (%) was determined by dividing the extension at rupture of the film by the initial length of the film (50 mm) multiplied by 100, and the EM (GPa) was determined from the slope of linear portion of the stress-strain curve, which corresponds to the stress divided by the strain of the film sample. Ten replicates were tested for each sample and the average values were presented.

Thickness of films was determined as average of five random positions of the film using a micrometer (Dial thickness gauge 7301, Mitutoyo, Japan) at an accuracy of 0.01 mm.

2.7. Water vapor permeability (WVP)

The water vapor permeability (WVP) of films was determined gravimetrically following the standard method of ASTM E96-95 with modification (Gennadios, Weller, & Goodings, 1994). The cup made of flaxy glass [poly(methylmethacrylate)] with an average

depth of 2.5 cm and inside diameter of 6.8 cm was used to measure WVP. Films were cut into rectangular shape (7.5 cm × 7.5 cm) and directly placed on the top of cups containing 18 mL of water. The each cup with film was tightened using screws to prevent leakage of water vapor. The entire cup was weighed and subsequently placed in a humidity chamber set at 25 °C and 50% RH. At every 1 h time interval, the weight loss from each cup was measured for 8 h. The slopes of the steady-state (linear) portion of weight loss versus time curves were used to calculate the water vapor transmission rate (WVTR; g/m² s) of the film. Then, the WVP (g m/m² s Pa) of the film was calculated as follows:

$$WVP = (WVTR \times L) / \Delta p \quad (4)$$

where L was mean thickness of the film (m) and Δp was partial water vapor pressure difference (Pa) across the film.

2.8. Water contact angle (CA)

Surface hydrophobicity of the film was determined by measuring water contact angle (CA) of the film surface using a CA analyzer (model Phoenix 150, Surface Electro Optics Co., Ltd., Kunpo, Korea). For this, films were cut into rectangular piece (3 cm × 10 cm) and directly placed on the horizontal movable stage (black Teflon coated steel, 7 cm × 11 cm) that fitted with the CA analyzer. A drop of water (ca. 10 μL) was placed on surface of the film using a micro syringe. The contact angle on both sides of the water droplet was measured to assume symmetry and horizontal level. Three measurements were taken for each sample and average values were presented as degree of CA.

2.9. Moisture content (MC) and water solubility (WS)

Moisture content (MC) of agar and agar/CNC films was determined using a drying oven method (Rhim & Wang, 2013). The rectangular films were cut into square of 3 cm × 3 cm and subsequently dried at 105 °C for 24 h using a drying oven. The MC was calculated from the weight loss and expressed as percent MC.

The water solubility of agar/CNC composite films was determined as the percentage of soluble matter of the film sample (Gontard, Guilbert, & Cuq, 1992). For this, three randomly selected specimens of each type of film were first dried at 105 °C for 24 h to determine the initial dry matter (W_1). Separate film sample were immersed in 30 mL of distilled water in a 50 mL beaker with gentle stirring. The film samples were removed after 24 h and dried in a drying oven at 105 °C for 24 h to determine the final dry weight (W_2). The WS of the sample was calculated as follows:

$$WS(\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (5)$$

2.10. Thermal stability

Thermal stability of mulberry pulp fiber, CNC, agar and agar/CNC composite films was determined by using a thermogravimetric analyzer (Hi-Res TGA 2950, TA Instrument, New Castle, DE, USA). For this, about 5 mg of samples were taken in standard aluminum cup and empty cup was used as a reference and heated from room temperature to 600 °C at heating rate of 10 °C/min under a nitrogen flow of 50 cm³/min. Derivative form of TGA (DTG) was obtained using differentials of TGA values, calculated using a central finite difference method as follows:

$$DTG = (w_{t+\Delta t} - w_{t-\Delta t}) / 2\Delta t \quad (6)$$

where $w_{t+\Delta t}$ and $w_{t-\Delta t}$ are the residual weight of sample at time $t + \Delta t$ and $t - \Delta t$, respectively, and Δt is the time interval for reading

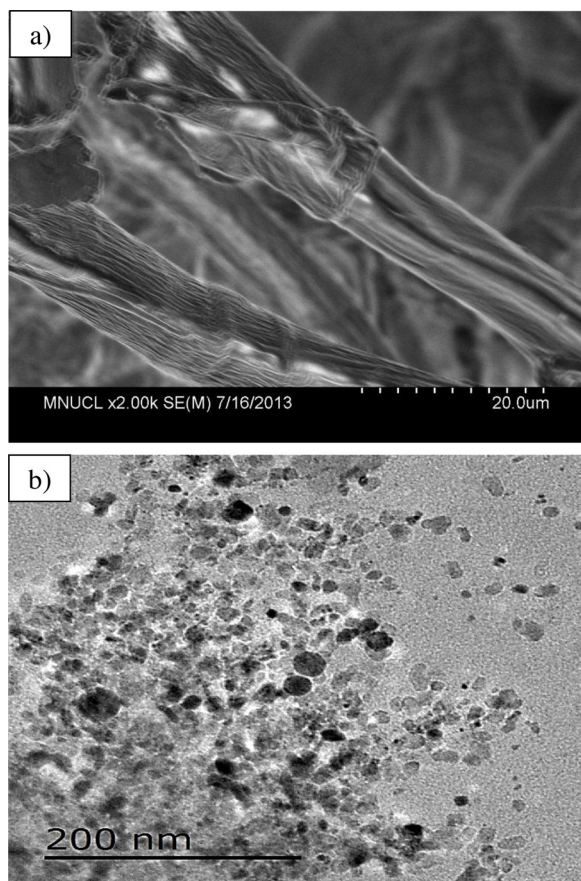


Fig. 1. Morphology of mulberry pulp fiber and its crystalline nanocellulose. (a) SEM image of mulberry pulp fiber; (b) TEM image of crystalline nanocellulose.

residual sample weight (Rhim, 2013). The maximum decomposition temperature ($T_{\max}$) of the agar and agar/CNC composite films was obtained from DTG curve and the char content and the weight loss (%) were measured using the TGA curve.

2.11. Statistical analysis

Measurements of each property of films were performed in triplicate with individually prepared film samples as the replicated experimental units, and mean values with standard deviations (SD) were reported. One-way analysis of variance (ANOVA) was performed, and the significance of each mean property value was determined ($p < 0.05$) with the Duncan's multiple range test using the SPSS statistical analysis computer program for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Morphology of mulberry fibers and its nanocellulose

Surface morphology of mulberry pulp fiber and its CNC was observed by FE-SEM and TEM, respectively. After beating, bundles of the pristine mulberry fiber were separated into single micro-sized fibers with a diameter of 40–70 μm (Fig. 1a). Li et al. (2009) also observed the individually separated cellulose fibers of mulberry bark with a diameter of 25 μm after treatment with NaOH solution. Crystalline nanocellulose (CNC) of mulberry pulp fiber with size reduced from micron to nanometer was obtained by removing the amorphous region of microfibrils through sulfuric acid hydrolysis (Li et al., 2009).

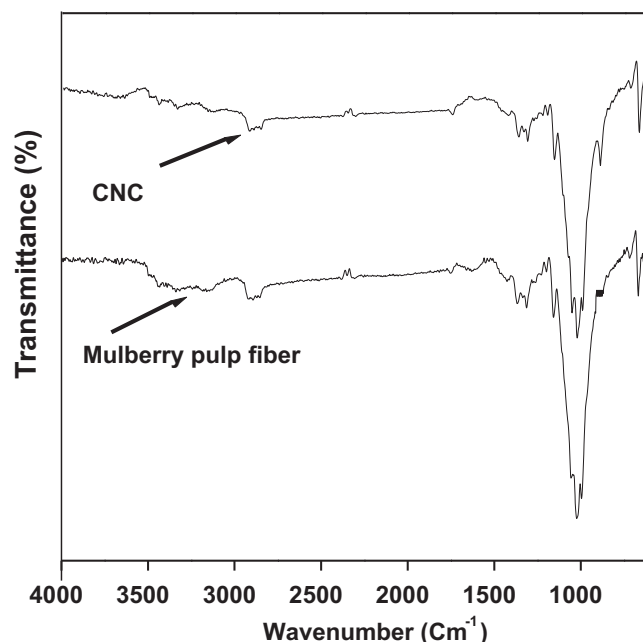


Fig. 2. FT-IR spectra of mulberry pulp fiber and its crystalline nanocellulose.

The TEM image of the CNC (Fig. 1b) exhibits uniformly dispersed individual crystallites. The mulberry pulp CNC was rod-like or spherical in shape with diameter of 40–50 nm and length of 200–350 nm. Li et al. (2009) separated mulberry nanocellulose by the acid hydrolysis method using 64% H_2SO_4 solution and they obtained nanowhiskers with a diameter of 20–40 nm. The difference in diameter of mulberry nanocellulose may be caused not only by the difference in the variety of raw materials, but also by the difference in the acid concentration used (64% vs. 47%). The higher acid solution tends to degrade the glucosidic linkages that constitute the whiskers, resulting in the decrease in their dimensions.

3.2. FT-IR analysis

The change in the chemical structure of mulberry fibers before and after acid hydrolysis was tested using FT-IR absorption spectroscopy and the results are shown in Fig. 2. The band at 3380 cm^{-1} corresponds to O–H stretching of the cellulose present in the fiber (Reddy, Maheswari, Reddy, Guduri, & Rajulu, 2010). The absorption peaks around 2908 cm^{-1} for native fiber and CNC are due to the stretching vibration of C–H groups of cellulose. The peak corresponds to 1372 cm^{-1} is due to the O–H bending vibration of cellulose (Li et al., 2012). The peak observed in the spectra of both samples at 1150 cm^{-1} is due to the C–O–C pyranose ring stretching vibration and the peak at 896 cm^{-1} indicates C–H rocking vibration of cellulose present in the microfibrils and nanofibrils (Alemdar & Sain, 2008a). The results of FT-IR showed that there was no distinctive change in absorption peak positions of the CNC compared with those of the mulberry fiber, which indicates that the acid hydrolysis of mulberry fiber did not affect the chemical structure of the cellulose fragments (Haafiz, Eichhorn, Hassana, & Jawaid, 2013). However, the intensity of peaks of the mulberry CNC was higher than those of native fiber, which indicates that the degree of crystallinity of CNC was higher than that of cellulose fiber (Noor, Ishak, & Ibrahim, 2003). This was further confirmed by the XRD analysis.

3.3. XRD analysis

The X-ray patterns of the mulberry pulp fiber and its CNC are shown in Fig. 3. Both diffractograms showed a peak at 2θ of 22.6°

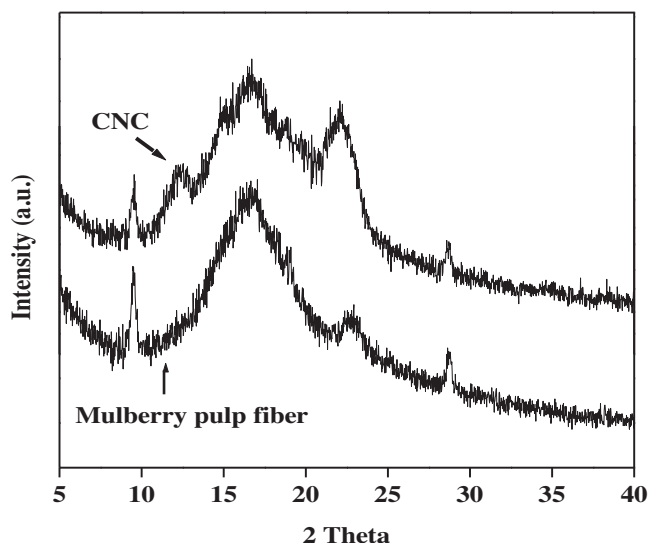


Fig. 3. XRD patterns of mulberry pulp fiber and its crystalline nanocellulose.

and a shoulder in the 2θ range of $14\text{--}17^\circ$, which represent typical cellulose I structure (Abeer, El-Wahab, Ibrahim, & Al-Shemy, 2010). However, the intensity of the peaks of the CNC was significantly higher than that of the mulberry fiber. The higher diffraction peak at 2θ of 22.6° of the CNC indicates that the degree of crystallinity of the CNC is greater than that of the mulberry fiber (Alemdar & Sain, 2008b). The degree of crystallinity of the mulberry fiber and its CNC estimated using Eq. (1) were 48.9% and 69.6%, respectively. The increased crystallinity of CNC was due to the removal of amorphous regions in the mulberry pulp fiber by the acid hydrolysis. Li et al. (2009) also observed the higher crystallinity of acid hydrolyzed cellulose nanowhiskers of mulberry bast fiber, and they reported that realignment of monocrystals during acid hydrolysis was responsible for the increased crystallinity. The crystallite size (calculated using Eq. (2)) of the mulberry pulp fiber and CNC found to be 117 and 129 nm respectively. It has been reported that the cellulose changes the size of the crystallites during acid hydrolysis (Maiti et al., 2013).

3.4. Thermal stability of mulberry fiber and nanocellulose

The thermal stability of mulberry pulp fiber and its CNC was tested using TGA and the results are shown in Fig. 4. Generally, two steps of thermal degradation of the mulberry fiber and its CNC was observed. The initial weight loss, caused by the moisture evaporation, was observed in the temperature range of $80\text{--}100^\circ\text{C}$. Then the main thermal degradation was occurred in the range of $200\text{--}370^\circ\text{C}$, which is mainly due to the thermal degradation of cellulosic materials. The TGA results exhibited that the thermal stability of the CNC was significantly lower than the mulberry fiber. This is presumably because more heat stable non-cellulosic materials in the fiber have been removed by acid hydrolysis. Li et al. (2009) also found that the thermal stability of cellulose nanowhisker isolated from mulberry

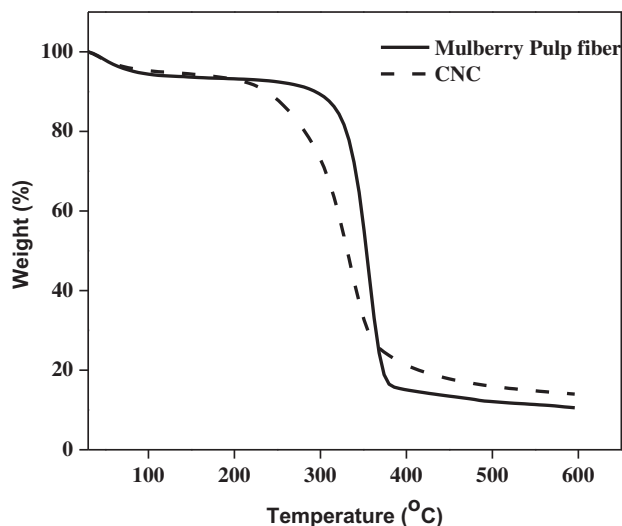


Fig. 4. TGA curves of mulberry pulp fiber and its crystalline nanocellulose.

bark using 64% H_2SO_4 solution was lower than its fiber counterpart. The decrease in thermal stability of the CNC may be further facilitated by the sulfated groups introduced to the nanocrystals during the sulfuric acid hydrolysis of the fiber (Li et al., 2009; Roman & Winter, 2004).

3.5. Apparent color and transparency of nanocomposite film

Apparently, agar and all the agar/CNC nanocomposite films prepared by the solvent casting method were free-standing and flexible as well as homogeneous. Apparent surface color values and transmittance of the agar film was affected by the inclusion of CNC as shown in Table 1. While Hunter L - and a -values (indicating lightness and green/redness, respectively) decreased slightly, Hunter b -value (indicating blue/yellowness) of the agar/CNC composite film increased with increase in the content of CNC, consequently, the total color difference value (ΔE) increased linearly with increase in the CNC content. Agar film was very transparent with transmittance ($T_{660\text{ nm}}$) of 87.7%. However, it decreased linearly with increase in the CNC content. The decrease in the transmittance of the agar/CNC nanocomposite films was attributed to the hindrance of light passage by the light impenetrable CNC particles. The linear decrease in the transmittance of the agar/CNC nanocomposite film could be expressed as following equation:

$$T = -3.16X + 86.58 \quad (R^2 = 0.99)$$

where T and X are transmittance of the film ($T_{660\text{ nm}}$) and content of CNC (%), respectively.

3.6. Surface morphology of nanocomposite films

The nanofiber distribution and the interaction between nanofiber and polymer matrix were observed by SEM analysis.

Table 1
Apparent color and transmittance of agar and agar/CNC composite films.^a

Film	L	a	b	ΔE	$T_{660\text{ nm}}$ (%)
Agar	92.6 ± 0.17^c	-0.56 ± 0.03^d	5.54 ± 0.01^a	3.52 ± 0.10^a	87.7 ± 0.4^e
Agar/CNF1%	92.1 ± 0.12^b	-0.63 ± 0.01^{bc}	5.60 ± 0.15^a	3.88 ± 0.07^b	83.7 ± 0.6^d
Agar/CNF3%	92.0 ± 0.25^b	-0.60 ± 0.01^c	5.60 ± 0.30^b	4.03 ± 0.12^b	76.1 ± 0.6^c
Agar/CNF5%	91.7 ± 0.12^b	-0.74 ± 0.01^a	6.70 ± 0.10^c	4.63 ± 0.09^c	69.4 ± 0.3^b
Agar/CNF10%	91.2 ± 0.36^a	-0.68 ± 0.03^b	7.10 ± 0.02^d	5.09 ± 0.18^d	55.9 ± 0.6^a

^a Each value is the mean of three replicates with the standard deviation. Any two means in the same column followed by the same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

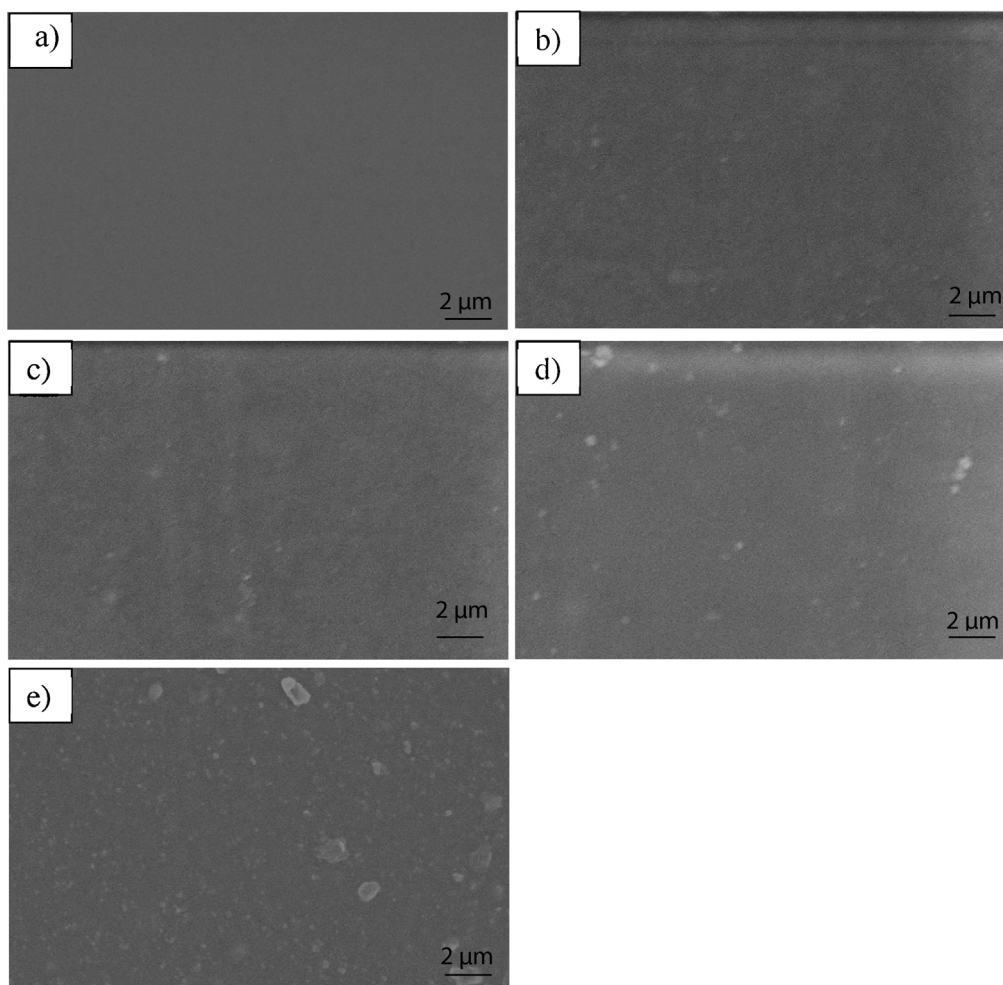


Fig. 5. SEM images of surface of agar and agar/CNC composite films. (a) Agar; (b) agar/CNC1%; (c) agar/CNC3%; (d) agar/CNC5%; (e) agar/CNC10%.

Fig. 5 shows the SEM images of the surface of agar and agar/CNC nanocomposite films. As shown in the SEM images, the pristine agar film exhibited smooth surface and the CNC particles in the composite films have been well dispersed in the polymer matrix though the degree of smoothness of the film surface was dependent on the CNC concentration. Up to the CNC concentration of 5 wt%, the surface of the nanocomposite films was as smooth as that of the pristine agar film, but the surface became less homogeneous when the CNC concentration increased to 10 wt%. This is mainly because of the agglomeration of CNC in the polymer matrix at high concentration (Agustin et al., 2013).

3.7. Mechanical properties of the composite films

Mechanical properties of agar and agar/CNC nanocomposite films are shown in Table 2. The thickness of CNC-included composite films increased significantly ($p < 0.05$) compared with that of the pristine agar film. In addition, it increased linearly with the concentration of CNC. This is due to the increase in the solid content of the film. The mechanical properties of agar film were also greatly changed after blending with the CNC and the properties were influenced with the amount of the CNC addition. The TS of agar film increased from 33.3 MPa of the pristine agar film up to 41.3 MPa with 3 wt% addition of the CNC then decreased with more addition of the CNC. However, the strength of the composite film with the highest amount of the CNC still comparable to that of the pristine agar film. This behavior may be attributed to

the crystalline nanocellulose with high aspect ratio (2–120 nm in diameter and 100-nm in length) and very high elastic modulus (130–250 GPa), which may induce a strong interfacial interaction through hydrogen or ionic bonds between the CNC and the polymer matrix (Siqueira et al., 2010). However, such reinforcing effect of the CNC was reduced at high level of CNC addition (10 wt%), probably due to the formation of aggregation of the CNC as observed in the SEM analysis. High amount of CNC may reduce the interaction between the CNC and polymer matrix due to the non-uniform stress distribution in the film, and consequently reduce the strength of the composite film. The tensile test result suggests that there is an optimum concentration of the filler to induce the maximum increment of the strength of the composite films. Similar mechanical performances have been reported for the addition of cellulose nanofibers

Table 2
Tensile properties of agar and agar/CNC composite films.^a

Film	Thickness (μm)	TS (MPa)	EM (GPa)	E (%)
Agar	55.7 ± 1.0^a	33.3 ± 2.5^a	0.77 ± 0.02^a	32.9 ± 1.0^b
Agar/CNC1%	60.1 ± 1.0^b	36.5 ± 1.8^b	0.82 ± 0.07^a	35.2 ± 2.1^c
Agar/CNC3%	64.4 ± 1.6^c	41.3 ± 1.5^d	0.91 ± 0.08^b	36.2 ± 1.6^c
Agar/CNC5%	71.4 ± 1.3^d	39.2 ± 1.4^c	0.99 ± 0.01^b	32.1 ± 1.5^b
Agar/CNC10%	80.1 ± 1.0^e	33.3 ± 2.2^a	1.11 ± 0.03^c	22.8 ± 1.3^a

^a Each value is the mean of three replicates with the standard deviation. Any two means in the same column followed by the same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

Table 3Moisture content, water vapor permeability, water contact angle and water solubility of agar and agar/CNC composite films.^a

Film	MC (% w.b.)	WVP ($\times 10^{-9}$ g m/m ² Pa s)	CA (deg.)	WS (%)
Agar	16.4 $\pm$ 0.4 ^c	2.11 $\pm$ 0.01 ^e	52.3 $\pm$ 0.6 ^d	28.6 $\pm$ 2.0 ^b
Agar/CNC1%	14.2 $\pm$ 0.8 ^{ab}	1.74 $\pm$ 0.01 ^b	48.9 $\pm$ 0.9 ^c	26.2 $\pm$ 0.5 ^a
Agar/CNC3%	13.6 $\pm$ 0.8 ^a	1.63 $\pm$ 0.02 ^a	47.2 $\pm$ 0.7 ^b	25.7 $\pm$ 0.2 ^a
Agar/CNC5%	14.4 $\pm$ 0.3 ^{ab}	1.79 $\pm$ 0.01 ^c	46.1 $\pm$ 0.9 ^{ab}	27.7 $\pm$ 0.6 ^{ab}
Agar/CNC10%	15.6 $\pm$ 1.2 ^{bc}	1.86 $\pm$ 0.02 ^d	44.4 $\pm$ 1.3 ^a	29.1 $\pm$ 1.2 ^b

^a Each value is the mean of three replicates with the standard deviation. Any two means in the same column followed by the same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

to other biopolymers such as starch and chitosan (Agustin et al., 2013; Azeredo et al., 2010; Wan et al., 2009).

The elastic modulus (EM) of the composite film, which measures degree of stiffness of a film, also increased linearly with the concentration of the CNC. The stiffness of the agar film increased 44% more than the pristine agar film when 10 wt% of the CNC was included. This again is mainly due to the nanofiller with high elastic modulus.

The elongation at break (E), which measures flexibility or ductility, of agar films also varied depending on the content of CNC. The pattern of change in the E of agar/CNC composite films was similar to that of the TS, namely, it increased with increase in the content of CNC up to 5 wt%, then decreased with further increase in the filler. The decrease in the flexibility of the composite film at high concentration of the filler may be due to the restricted mobility of polymer strand caused by the increased stiffness of the film.

3.8. Water vapor permeability (WVP), water contact angle (CA), and water solubility (WS) of the composite films

The results on WVP of agar and agar/CNC composite films are presented in Table 3. The WVP of the pristine agar film was 2.11×10^{-9} g m/m² s Pa, and it decreased significantly ($p < 0.05$) after formation of nanocomposite with the CNC. However, the decrease in the WVP of the composite film was also dependent on the concentration of the nanofiller. It decreased significantly with increase in the content of CNC down to 1.63×10^{-9} g m/m² s Pa (decrease in 23%) with 3 wt% of CNC inclusion, then it increased with further increase in the nanofiller content. It is interesting to note that 3 wt% of CNC was also the optimum concentration to improve the water vapor barrier property of agar film as in the case of improvement of the TS of the film. Generally, the water vapor barrier property of composite film is improved when less permeable fillers are well dispersed in the polymer matrix (Rhim et al., 2011). The impermeable CNC that dispersed in the polymer matrix would form a tortuous path for water vapor diffusion, and consequently increase the effective diffusion path length resulting in the decrease of the WVP (Rhim, Hong, & Ha, 2009). However, the WVP increased after 3 wt% of CNC loading. This might be due to the voids the agar/CNC composite mainly caused by the agglomeration of the CNC in the polymer matrix as shown in the SEM analysis.

The impermeable nanofillers in the polymer matrix cause a tortuous pathway for water vapor diffusion leads to slower diffusion processes and hence results in a lower WVP. The cellulose nanocrystal in the agar/CNC composite films may also act as a barrier for the diffusion of water vapor in the polymer matrix to decrease the WVP of the composite films.

The CA is usually used as a measure of wettability or surface hydrophobicity of polymer films. The CA of agar and agar/CNC composite films is also shown in Table 3. The surface hydrophobicity of the agar/CNC composite films was significantly ($p < 0.05$) lower than that of the pristine agar film. In addition, the CA of agar/CNC composite film decreased monotonously with increase in the CNC concentration. This might be attributed to that the cellulose nanofiller is more hydrophilic than the base polymer. Hydrophilicity of the CNC may have been increased by the

exclusion of non-polar components, the insertion of polar sulfate groups, and the exposure of hydroxyl groups from the cellulose structure during the isolation of the CNC (Morais et al., 2013). Bahar et al. (2012) also found that the inclusion of cellulose nanowhiskers in polypropylene composite films decreased contact angle of the film.

Table 3 also shows the result of WS of the agar and agar/CNC composite films. The WS of agar films was also affected by the inclusion of the CNC. The WS of agar/CNC composite film decreased from 28.6% of the pristine agar film down to 25.7% of agar/CNC composite film with 3 wt% inclusion of CNC, and it increased with further inclusion of the CNC. This result indicates that the agar/CNC composite

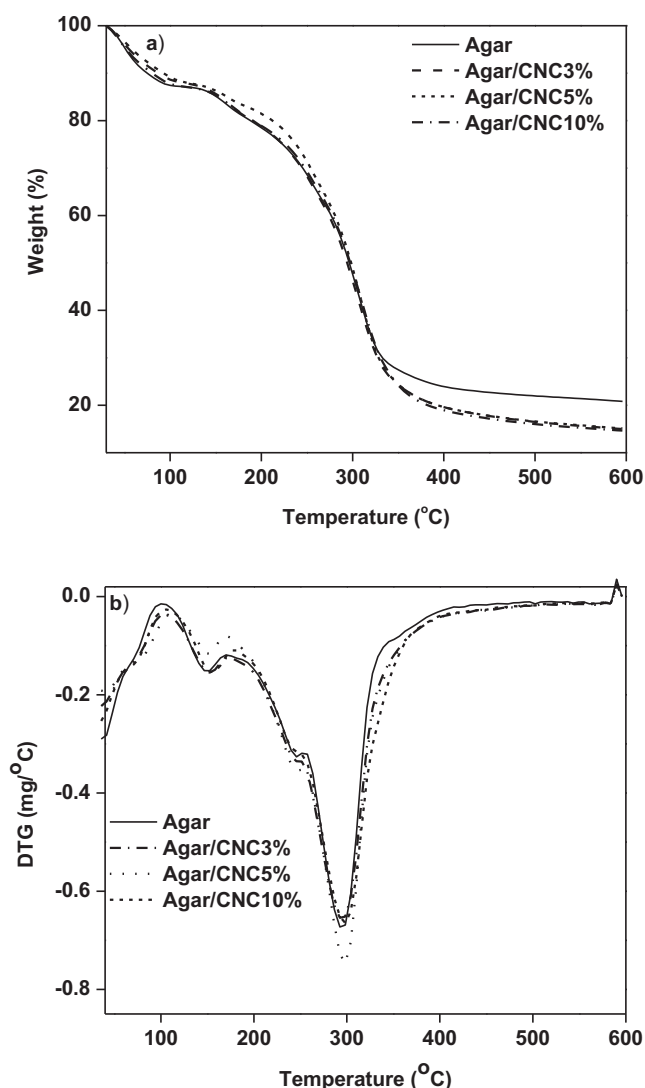


Fig. 6. TGA and DTG curves of agar and agar/CNC composite films.

Table 4
Results of thermogravimetric analysis of bionanocomposites.

Film	$T_{\max}$ (°C) ^a	Decomposition (%)	Char at 600 °C (%)
Agar	152	15.2	20.9
	251	31.9	
	298	52.1	
Agar/CNC3%	158	15.5	15.4
	257	32.2	
	304	56.4	
Agar/CNC5%	159	14.6	15.1
	256	31.8	
	304	54.1	
Agar/CNC10%	158	15.7	14.6
	253	31.4	
	304	54.2	

^a The maximum decomposition temperature at each decomposition step.

film reached its maximum water resistance when 3 wt% of CNC was included.

3.9. Thermal stability of the composite films

The thermal stability of agar and agar/CNC composites films were tested using TGA, and their TGA and DTGA curves were shown in Fig. 6. The TGA curves show weight decreasing pattern of film due to the thermal degradation and the DTGA curves show the maximum decomposition temperature ($T_{\max}$) at each stage of thermal degradation (Rhim, 2013). The results of TGA and DTGA curves for the thermal degradation of agar and agar/CNC composite films were summarized in Table 4. The TGA of agar and agar/CNC composite films exhibited three distinctive weight loss regions. The first region at a temperature of 80–150 °C was due to the evaporation of moisture (physically weak and chemically strong bound water) with the weight loss of about 15 wt%. The second degradation occurred at around 190–250 °C, which was attributed to volatilization of glycerol used for plasticizer. The third and main stage of weight loss occurred above 300 °C, which was due to the thermal degradation of the biopolymer chain.

Generally, the agar/CNC composite films exhibited slightly higher thermal stability than the pristine agar film up to the second stage of thermal degradation, though it was not so significant. Char at 600 °C, which is the amount of residue left after final thermal decomposition, of the agar/CNC composite films was significantly less than that of the pristine agar film. The char at 600 °C of agar film was 20.9%, but it decreased down to 15.4–14.6% depending on the CNC concentration of the agar/CNC composite films. This is mainly due to higher ash content of agar than the CNC. Similar thermal degradation behavior has been observed in the nanocrystalline cellulose reinforced chitosan films (Khan et al., 2012).

4. Conclusions

Crystalline nanocellulose (CNC) was isolated from paper mulberry pulp fiber using sulfuric acid hydrolysis method and the CNC was used to prepare agar/CNC composite films with varying concentration of the CNC. The results revealed that the film properties such as mechanical and water vapor barrier properties have been significantly improved with lower amount of the CNC inclusion (less than 5 wt%). This was due to the reinforcement of high crystalline CNC caused by the strong interaction between the homogeneously dispersed CNC and the polymer matrix. The agar/CNC bionanocomposite film, which is completely biodegradable and biocompatible, has a high potential to be used for food packaging or biomedical applications.

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References

- Abdul Khalil, H. P. S., Bhat, A. H., & Ireana Yusra, A. F. (2012). Green composites from sustainable cellulose nanofibrils: A review. *Carbohydrate Polymers*, 87, 963–979.
- Abeer, M. A., El-Wahab, Z. H. A., Ibrahim, A. A., & Al-Shemy, M. T. (2010). Characterization of microcrystalline cellulose prepared from lignocellulosic materials. Part I. Acid catalyzed hydrolysis. *Bioresource Technology*, 101, 4446–4455.
- Agustin, M. B., Ahmmad, B., Richel, E. P. D. L., Jerico, L. B., Joel, R. S., & Fumihiko, H. (2013). Starch-based biocomposite films reinforced with cellulose nanocrystals from garlic stalks. *Polymer Composites*, 34, 1325–1332.
- Alemdar, A., & Sain, M. (2008a). Isolation and characterization of nanofibers from agricultural residues-wheat straw and soy hulls. *Bioresource Technology*, 99, 1664–1671.
- Alemdar, A., & Sain, M. (2008b). Biocomposites from wheat straw nanofibers: Morphology, thermal and mechanical properties. *Composites Science and Technology*, 68, 557–565.
- Azeredo, H. M. C., Mattoso, L. H. C., Avena-Bustillos, R. J., Filho, G. C., Munford, M. L., Wood, D., et al. (2010). Nanocellulose reinforced chitosan composite films as affected by nanofiller loading and plasticizer content. *Journal of Food Science*, 75(1), N1–N7.
- Azizi Samir, M. A. S., Alloin, F., & Dufresne, A. (2005). A review of recent research into cellulosic whiskers, their properties and their application in nanocomposite field. *Biomacromolecules*, 6, 612–626.
- Bahar, E., Ucar, N., Onen, A., Wang, Y., Oksu, M., Ayaz, O., et al. (2012). Thermal and mechanical properties of polypropylene nanocomposite materials reinforced with cellulose nanowhiskers. *Journal of Applied Polymer Science*, 125, 2882–2889.
- Da Silva, J. B. A., Pereira, F. V., & Druzian, J. I. (2012). Cassava starch-based films plasticized with sucrose and inverted sugar and reinforced with cellulose nanocrystals. *Journal of Food Science*, 77, N14–N19.
- Das, K., Ray, D., Bandyopadhyay, N. R., Ghosh, T., Mohanty, A. K., & Misra, M. (2009). A study of the mechanical, thermal and morphological properties of microcrystalline cellulose particles prepared from cotton slivers using different acid concentrations. *Cellulose*, 16, 783–793.
- De Souza Lima, M. M., & Borsali, R. (2004). Rodlike cellulose microcrystals: Structure properties and applications. *Macromolecular Rapid Communications*, 25, 771–787.
- Faruk, O., Bledzki, A. K., Fink, H. P., & Sain, M. (2012). Biocomposites reinforced with natural fibers: 2000–2010. *Progress in Polymer Science*, 37, 1552–1596.
- Gennadios, A., Weller, C. L., & Goodings, C. H. (1994). Measurement errors in water vapor permeability of high permeable hydrophilic edible films. *Journal of Food Engineering*, 21, 395–409.
- Gontard, N., Guilbert, S., & Cuq, J. L. (1992). Edible wheat gluten films: Influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*, 57, 190–195.
- Gumuskaya, E., Usta, M., & Kirci, H. (2003). The effects of various pulping conditions on crystalline structure of cellulose in cotton linters. *Polymer Degradation and Stability*, 81, 559–564.
- Haafiz, M. K. M., Eichhorn, S. J., Hassana, A., & Jawaaid, M. (2013). Isolation and characterization of microcrystalline cellulose from oil palm biomass residue. *Carbohydrate Polymers*, 93, 628–634.
- Huq, T., Salmieri, S., Khan, R. A., Tien, C. L., Riedl, B., Frascini, C., et al. (2012). Nanocrystalline cellulose (NCC) reinforced alginate based biodegradable nanocomposite film. *Carbohydrate Polymers*, 90, 1757–1763.
- Jawaaid, M., & Abdul Khalil, H. P. S. (2011). Cellulosic/synthetic fibre reinforced polymer hybrid composites: A review. *Carbohydrate Polymers*, 86, 1–18.
- John, M. J., & Thomas, S. (2008). Biofibres and biocomposites. *Carbohydrate Polymers*, 71, 343–364.
- Khan, A., Huq, T., Khan, R. A., Riedl, B., & Lacroix, M. (2014). Nanocellulose-based composites and bioactive agents for food packaging. *Critical Reviews in Food Science and Nutrition*, 54, 163–174.
- Khan, A., Khan, R. A., Salmieri, S., Tien, C. L., Riedl, B., Bouchard, J., et al. (2012). Mechanical and barrier properties of nanocrystalline cellulose reinforced chitosan based nanocomposite films. *Carbohydrate Polymers*, 90, 1601–1608.
- Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D., et al. (2011). Nanocelluloses: A new family of nature-based materials. *Angewandte Chemie International Edition*, 50, 5438–5466.
- Lavoine, N., Desloges, I., Dufresne, A., & Bras, J. (2012). Microfibrillated cellulose – Its barrier properties and applications in cellulosic materials: A review. *Carbohydrate Polymers*, 90, 735–764.
- Li, R., Fei, J., Cai, Y., Li, Y., Feng, J., & Yao, J. (2009). Cellulose whiskers extracted from mulberry: A novel biomass production. *Carbohydrate Polymers*, 76, 94–99.
- Li, J., Wei, X., Wang, Q., Chen, J., Chang, G., Kong, L., et al. (2012). Homogeneous isolation of nanocellulose from sugarcane bagasse by high pressure homogenization. *Carbohydrate Polymers*, 90, 1609–1613.

- Lim, G. H., & Choi, T. H. (2008). The effects of the cutting length of paper mulberry bast fiber on pulping and Hanji properties (I) – White bast of Korea grown paper mulberry. *Journal of Korea TAPPI*, 40, 57–64.
- Maiti, S., Jayaramudu, J., Das, K., Reddy, S. M., Sadiku, R., Ray, S. S., et al. (2013). Preparation and characterization of nano-cellulose with new shape from different precursor. *Carbohydrate Polymers*, 98, 562–567.
- Morais, J. P. S., Rosa, M. F., Filho, M. M. S., Nascimento, L. D., Nascimento, D. M., & Cassales, R. (2013). Extraction and characterization of nanocellulose structures from raw cotton linter. *Carbohydrate Polymers*, 91, 229–235.
- Noor, A. R., Ishak, A., & Ibrahim, A. (2003). Isolation and characterization of cellulose nano crystals from agave angustifolia fiber. *BioResources*, 8, 1893–1908.
- Peng, B. L., Dhar, N., Liu, H. L., & Tam, K. C. (2011). Chemistry and applications of nanocrystalline cellulose and its derivatives: A nanotechnology perspective. *Canadian Journal of Chemical Engineering*, 89, 1191–1206.
- Qu, C., & Wang, S. (2011). Macro-micro structure, antibacterial activity and physico-mechanical properties of the mulberry bast fibers. *Fiber and Polymers*, 12, 471–477.
- Reddy, M. M., Vivekanandhan, S., Misra, M., Bhatia, S. K., & Mohanty, A. K. (2013). Biobased plastics and bionanocomposites: Current status and future opportunities. *Progress in Polymer Science*, 38, 1653–1689.
- Reddy, K. O., Maheswari, C. U., Reddy, D. J. P., Guduri, B. R., & Rajulu, A. V. (2010). Properties of ligno-cellulose ficus religiosa leaf fiber. *International Journal of Polymers and Technologies*, 2, 29–35.
- Rhim, J. W. (2011). Effect of clay content on mechanical and water vapor barrier properties of agar-based nanocomposite films. *Carbohydrate Polymers*, 86, 691–699.
- Rhim, J. W. (2013). Effect of PLA lamination on performance characteristics of agar/ κ -carrageenan/clay bio-nanocomposite film. *Food Research International*, 51, 714–722.
- Rhim, J. W., & Wang, L. F. (2013). Mechanical and water barrier properties of agar/ κ -carrageenan/konjac glucomannan ternary blend biohydrogel films. *Carbohydrate Polymers*, 96, 71–81.
- Rhim, J. W., Lee, S. B., & Hong, S. I. (2011). Preparation and characterization of agar/clay nanocomposite films: The effect of clay type. *Journal of Food Science*, 76, N40–N48.
- Rhim, J. W., Hong, S. I., & Ha, C. S. (2009). Tensile, water barrier and antimicrobial properties of PLA/nanoclay composite films. *LWT – Food Science and Technology*, 42, 612–617.
- Roman, M., & Winter, W. T. (2004). Effect of sulfate groups from sulfuric acid hydrolysis on the thermal degradation behavior of bacterial cellulose. *Biomacromolecules*, 5, 1671–1677.
- Siqueira, G., Bras, J., & Dufresene, A. (2010). Cellulosic bionanocomposites: A review of preparation properties and applications. *Polymers*, 2, 728–765.
- Siró, I., & Plackett, D. (2010). Microfibrillated cellulose and new nanocomposite materials. *Cellulose*, 17, 459–494.
- Sorrentino, A., Gorrasi, G., & Vittoria, V. (2007). Potential perspectives of bionanocomposites for food packaging applications. *Trends in Food Science and Technology*, 18, 84–95.
- Tang, X. Z., Kumar, P., Alavi, S., & Sandeep, K. P. (2012). Recent advances in biopolymers and biopolymer-based nanocomposites for food packaging materials. *Critical Reviews in Food Science and Nutrition*, 52, 426–442.
- Walia, Y. K. (2013). Chemical and physical analysis of *Morus nigra* (Black Mulberry) for its pulpability. *Asian Journal of Advanced Basic Sciences*, 1, 40–44.
- Wan, Y. Z., Luo, H., He, F., Liang, H., Huang, Y., & Li, X. L. (2009). Mechanical, moisture absorption and biodegradation behaviours of bacterial cellulose fibre-reinforced starch biocomposites. *Composites Science and Technology*, 69, 1212–1217.