Transparent bionanocomposite films based on chitosan and TEMPO-oxidized cellulose nanofibers with enhanced mechanical and barrier properties

Bhawna Soni^a, El Barbary Hassan^{a*}, M. Wes Schilling^b, and Barakat Mahmoud^{b,c}

^aDepartment of Sustainable Bioproducts, Mississippi State University, Box 9820, Mississippi State, MS 39762, USA

^bDepartment of Food Science, Nutrition & Health Promotion, Mississippi State University, Box 9805, MS 39567, USA

^cDepartment of Food Science, Nutrition & Health Promotion and Coastal Research & Extension Center, Mississippi State University, 3411 Frederic St., Pascagoula, MS 39567, USA

E-mail address: e.hassan@msstate.edu

Abstract

The development of biobased active films for use in food packaging is increasing due to low cost, environmental appeal, renewability and availability. The objective of this research was to develop an effective and complete green approach for the production of bionanocomposite films with enhanced mechanical and barrier properties. This was accomplished by incorporating TEMPO-oxidized cellulose nanofibers (2,2,6,6-tetramethylpiperidine-1-oxyl radical) into a chitosan matrix. An aqueous suspension of chitosan (100–75 wt %), sorbitol (25 wt %) and TEMPO-oxidized cellulose nanofibers (TEMPO-CNFs, 0–25 wt %) were cast in an oven at 40 °C for 2-4 days. Films were preconditioned at 25°C and 50% RH for characterization. The surface morphology of the films was revealed by scanning electron microscopy (SEM) and atomic force

microscopy (AFM). The thermal properties and crystal structure of the films were evaluated by thermogravimetric analysis (TGA-DTG) and X-ray diffraction (XRD). Incorporation of TEMPO-CNFs enhanced the mechanical strength of the films due to the high aspect ratio (3–20 nm width, and 10–100 nm length) of TEMPO-CNFs and strong interactions with the chitosan matrix. Oxygen and water vapor transmission rates for films that are prepared with chitosan and TEMPO-CNFs (15–25 wt %) were significantly reduced. Furthermore, these bionanocomposite films had good thermal stability. Use of TEMPO-CNFs in this method makes it possible to produce bionanocomposite films that are flexible, transparent, and thus have potential in food packaging applications.

Keywords

Chitosan, Cellulose nanofibers, TEMPO-mediated oxidation, Oxygen permeability, Mechanical properties.

1. Introduction

Since the 1980s, plastic debris has accumulated on the earth due to the production and use plastic packaging that is not biodegradable (Thompson, Moore, vom Saal & Swan, 2009). This has contributed to environmental issues caused by non-biodegradable waste materials and the depletion of natural resources. Approximately more than 300 million tons of plastic are produced throughout the world in 2010 (Thompson, Moore, vom Saal & Swan, 2009). Packaging is the largest single market of plastics. In addition plastic disposal contributes to growing landfills and enhanced greenhouse effects when plastics are burned (Rhim & Ng, 2007). With the growing consumer demand for high quality food products and concern for limited natural

resources, researchers have investigated the use of biobased materials that are environmentally friendly, biodegradable, renewable, and abundant in nature. These biobased materials are being investigated for their feasibility to replace petrochemical based packaging such as, polyolefins and polyesters with a variety of renewable biopolymers. Polysaccharides (Krochta & Mulder-Johnston, 1997), proteins (Cuq, Gontard & Guilbert, 1998), lipids (Gennadios, Ghorpade, Weller & Hanna, 1996), and their composites (Bourtoom, 2008) can be used to produce biodegradable packaging that can maintain product quality and reduce waste disposal problems. Water vapor and oxygen permeability, mechanical strength, transparency, and flexibility are important properties for packaging films. Therefore, film formation requires the use of at least one component that is capable of forming a structural matrix with a sufficient cohesiveness. The degree of cohesion of the polymer matrix affects film properties such as density, permeability and porosity, brittleness and flexibility (Rhim & Ng, 2007). Previous research has evaluated chitosan (Martínez-Camacho et al., 2010), starch (Ghanbarzadeh, Almasi & Entezami, 2011), hemicellulose (Hansen & Plackett, 2008), xylan (Saxena, Elder & Ragauskas, 2011), cellulose (Wu et al., 2016), and their derivatives for film forming properties.

Chitosan is a natural polycationic, deacetylated derivative of chitin, which is found in the crustacean's shell, insect's cuticle and cell wall of fungi. It is a linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy- β -d-glucan, which is the second most abundant polysaccharide found in nature after cellulose (Dutta, Dutta & Tripathi, 2004). Chitosan exhibits unique physicochemical properties including biocompatibility, non-toxicity, biodegradability, excellent film forming ability and strong antimicrobial and antifungal

activities (Darmadji & Izumimoto, 1994; Jo, Lee, Lee & Byun, 2001). Chitosan has been evaluated for its applicability in biotechnology, pharmaceuticals, biomedicine, packaging, wastewater treatment, cosmetics, and food science among others (Belgacem & Gandini, 2011). Chitosan has been used to produce active biodegradable films to extend the shelf-life of food and prevent contamination. Chitosan may be more advantageous than other biomolecule based active films due to its antimicrobial activity and ability to chelate bivalent minerals (Chen, Zheng, Wang, Lee & Park, 2002). However, despite the numerous advantages and unique properties of chitosan, its films are poor barriers to gas and water vapor and have poor mechanical properties, which restricts its use in packaging applications. Therefore, several strategies have been used to improve the mechanical and barrier properties of chitosan films, including blending chitosan with polyvinyl alcohol (Li, Chen & Wang, 2015), poly *N*-vinyl pyrrolidone (Sakurai, Maegawa & Takahashi, 2000), polyethylene glycol (Kiuchi, Kai & Inoue, 2008), polyethylene oxide (Amiji, 1995), starch (Bourtoom & Chinnan, 2008), collagen (Sionkowska, Wisniewski, Skopinska, Kennedy & Wess, 2004), cellulose (Dehnad, Mirzaei, Emam-Djomeh, Jafari & Dadashi, 2014; Fernandes et al., 2009; Fernandes, Freire, Silvestre, Pascoal Neto & Gandini, 2011; Shih, Shieh & Twu, 2009), carboxymethyl cellulose (Dayarian, Zamani, Moheb & Masoomi, 2014) sulfonated cellulose fibers (Li, Chen & Wang, 2015), hydroxypropylmethylcellulose (Sebti, Chollet, Degraeve, Noel & Peyrol, 2007), and cellulose acetate (Abou-Zeid, Waly, Kandile, Rushdy, El-Sheikh & Ibrahim, 2011). Polymer blending is the most effective method to create biobased films with desirable properties.

The formulation of composite films from the utilization of green materials contributes to a more sustainable society. Chitosan-cellulose combinations are compatible due to structural similarity, which results in fibers with the physicochemical properties of chitosan and the mechanical properties of cellulose fibers (Twu, Huang, Chang & Wang, 2003; Wu et al., 2004; Yin, Luo, Chen & Khutoryanskiy, 2006). Cellulose is used to produce potential reinforcing bionanomaterials called cellulose nanofibers (Soni, Hassan & Mahmoud, 2015). Cellulose nanofibers (CNFs) have been researched for their use in biodegradable packaging due to their renewable, low cost, low density, and nonabrasive nature. This allows them to formulate bio-based nanocomposites with easy process ability. All these important characteristics of CNFs have made it an attractive candidate for nanomaterial research fields. More recently, cellulose of hardwood pulp (Shih, Shieh & Twu, 2009) and nanocellulose (Dehnad, Mirzaei, Emam-Djomeh, Jafari & Dadashi, 2014) have been incorporated into chitosan matrices to produce bionanocomposite films. Similarly, cellulose nanowhiskers (CNWs) and cellulose nanocrystals (CNCs) have been reinforced into chitosan biopolymers to produce green nanocomposite films, with improved thermal, mechanical and oxygen barrier properties (de Mesquita, Donnici & Pereira, 2010; El Miri et al., 2015; Li, Chen & Wang, 2015). However, in these research projects, water vapor permeability and transparency of the films was not evaluated. Furthermore, bacterial cellulose (Fernandes et al., 2009) incorporated into chitosan matrix have shown reasonable thermal, and mechanical properties but have not shown any concern about moisture permeability and oxygen barrier quality. Polysaccharides are generally good oxygen barriers because hydrogen bonds contribute to excellent packing of the material and thus a low permeability. Water

vapor and oxygen resistivity are fundamental requirement for new packaging materials since water molecules contribute microbial growth and influence the shelf life of the packaged products at different water activities and temperatures. Oxygen promotes degradation mechanisms in food such as corrosive phenomena, oxidations, and great modification of organoleptic properties (Russo, Simon & Incarnato, 2006). The tensile strength is an important factor in the film forming processes since brittle films are more likely to fracture. Thus testing tensile strength is a good way to measure how strong a material will be under certain conditions. In a long run, it is necessary to ensure a safe, high quality material and avoiding the major liabilities associated with providing non-compliant products. These actions will keep the end consumer satisfied and dramatically reduce the chance of failure of the packaging.

Recently, completely individualized cellulose nanofibers have been extracted from cotton stalks (Soni, Hassan & Mahmoud, 2015) by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation under moderate aqueous condition (Saito, Kimura, Nishiyama & Isogai, 2007). This oxidation process coupled with ultrasonication selectively converts primary alcohols to aldehyde and carboxylate groups (Soni, Hassan & Mahmoud, 2015). The width and length of TEMPO-CNFs was ranging from 3–15 nm and 10–100 nm of range. This was the first time to develop uniform nano-sized fibers, which are not only small in diameter but also small in length with more than 90% yield from cotton stalks (Soni, Hassan & Mahmoud, 2015). However, to the best of our knowledge, there are no studies about the blending of TEMPO-oxidized cellulose nanofibers and chitosan for producing bionanocomposite films with enhanced comprehensive performance for packaging applications. Hydrogen bonds and

electrostatic attraction between the negatively charged carboxylate groups ($-\text{COO}^-$) and the positively charged ammonium groups ($-\text{NH}_3^+$) of chitosan are the driving force for the best combination of both the polymers (Scheme 1). Therefore, these strong bonds ensure the high compatibility between both TEMPO-CNFs and the chitosan biopolymers.

The aim of this research is to describe a fully green approach for the preparation of transparent and high performance bionanocomposite films based on chitosan matrices with TEMPO-oxidized cellulose nanofibers as reinforcing agents. Extensive characterization of the chitosan/TEMPO-CNFs films revealed the unique properties of these films.

2. Experimental

2.1 Materials

Chitosan (DDA ~ 72%) was synthesized from Mississippi gulf brown shrimp (*penaeus aztecus*) exoskeletons, which were obtained from the Mississippi Gulf Coast in Biloxi, MS. Cellulose was isolated from cotton stalks by alkaline-acid pretreatment and used for the preparation of nanocellulose by the TEMPO-mediated oxidation method (Soni, Hassan & Mahmoud, 2015). Morphological (FE-SEM, AFM), structural (FTIR), and thermal analysis (TGA-DTG) of TEMPO-CNFs were investigated in our previous work (Soni, Hassan & Mahmoud, 2015). All chemicals used in this study were purchased from commercial resources and used as received without further purification. The following chemicals were purchased from Fisher Scientific, USA: ethanol (95%), glacial acetic acid, calcium sulfate, calcium nitrate, magnesium nitrate hexahydrate,

160 potassium sulfate, sorbitol, and TEMPO [(2,2,6,6-tetramethylpiperidin-1-yl) oxy radical].
 161 Teflon petri dish (140 mm×140 mm) liner was purchased from Fluoro Lab, USA.

162
 163 **Scheme 1.** The schematic representation of intermolecular hydrogen bonds and
 164 electrostatic interactions in chitosan/TEMPO-CNFs nanocomposite film.

165 2.2. Casting of Chitosan/TEMPO-CNFs films

166 Films were prepared by mixing chitosan, TEMPO-CNFs, and sorbitol in 2% glacial
 167 acetic acid solution for 1 h at 70 °C. The total amount of dry substance (chitosan,
 168 sorbitol, and TEMPO-CNFs) in each film was maintained at 1.25 g. The TEMPO-CNFs

contents in the nanocomposite films were 0, 5, 10, 15, 20, and 25 wt % of the total dry mixture, respectively. Sorbitol was kept constant at 25.0 wt % (based on the dry weight of chitosan and TEMPO-CNFs). Vacuum was applied to degas the film solutions to prevent microbubble formation. Solutions were poured onto 140 mm ×140 mm petri dish liners and dried in an oven at 40 °C for 2–4 days. It was then peeled carefully from the petri dish.

2.3. Characterization of films

2.3.1. Film thickness and conditioning

Before characterization, all film samples were preconditioned for at least 48 h in a constant temperature and humidity chamber (25 °C and 50% relative humidity (RH)) to ensure the stabilization of their water content. Film thickness was determined using a Fowler & NSK Max Cal electronic digital caliper. Ten measurements from each film were taken and reported as the average. These measurements were used in further analysis for water vapor transmission rate, oxygen transmission rate, and tensile strength.

2.3.2. Field emission scanning electron microscopy (FE-SEM)

Surface morphology of chitosan/TEMPO-CNFs blended films were examined before and after tensile strength investigation using FE-SEM (JEOL JSM-6500F Field Emission Scanning Electron Microscope). Films were mounted on aluminum specimen stubs using double adhesive carbon tapes and subjected directly to a high-performance scanning electron microscope to take the micrographs. Fractured surfaces after the tensile test were placed onto stubs (45/90°) and coated with 10 nm platinum that was

sprayed in EMS 150T ES sputter coater for fine grain sputtering, and then observed under FE-SEM. Micrographs were taken at an accelerating voltage of 5.0–10 kV.

2.3.3. Atomic force microscopy (AFM)

Topography and nanomorphology of film surfaces were studied using a Dimension Icon AFM ScanAsyst Bruker (USA) instrument. Prior to examination, small pieces of film were glued onto AFM specimen metal disks using Lift-N-Press™ Tabs and fixed to a magnetic sample holder located on the top of the scanner tube using a vacuum. Topographic (height) and phase images were scanned in tapping mode using a 1–10 Ω cm phosphorus (*n*) doped rectangular silicon cantilever from Veeco probes (MMP-11100-10). The resonance frequency was between 276–317 kHz, and images were recorded under ambient air conditions.

2.3.4. X-ray diffraction (XRD)

The X-ray diffraction pattern for each of the all chitosan/TEMPO-CNFs film (Preconditioned at 50% RH) was measured using a Rigaku smartlab X-ray diffractometer (Ultima III XRD, Rigaku Americas, The Woodlands, TX) to evaluate crystallinity in the film. Samples were analyzed in continuous mode in the angular range of 3°– 60° (2θ) with step increment of 0.02° (2θ). The Cu K α radiation generated at 40 kV and 44 mA was monochromatized using the K β filter method at the wavelength of λ = 0.15418 nm.

2.3.5. Optical Transmittance

The transmittance spectra of the films were scanned from 200 to 800 nm wavelength using Cary 100 Bio UV–vis Spectrophotometer (Varian Australia, Australia). Transmittance was measured by inserting a slide of each film in the cuvette stand, so

the beam was oriented at 90° with respect to the slide. The measurement was done in triplicate.

2.3.6. Moisture absorption

Moisture absorption was measured according to Angles and Dufresne (Angles & Dufresne, 2000). Four pieces of each film were cut into 2 cm × 2 cm squares and conditioned at 0% RH for 24 h. Samples were then weighed and placed in a desiccator at 55% RH. The relative humidity was created with a saturated solution of calcium nitrate at 22–25 °C. The samples were weighted at desired intervals until the equilibrium state was reached. The moisture absorption was calculated using the following equation:

$$\text{Moisture absorption (\%)} = \frac{W_t - W_o}{W_o} \times 100 \quad (1)$$

where, W_t is the weight of the sample after t time at 55% RH and W_o is the initial weight of the sample. Four replicate measurements were taken for each film.

2.3.7. Water solubility

Water solubility of each film was determined as the percentage of dry matter of the film solubilized after 24 h of immersion in distilled water. Film samples from each type of film were cut and dried at 105 °C for 24 h to determine the weight of the initial dry matter. Approximately 200 mg of each film sample was immersed in a beaker containing 20 mL of distilled water for 24 h at 23 °C with periodical, gentle stirring. The samples were removed from water and undissolved dry matter was determined by drying in an oven at 105 °C until a constant weight was obtained. The percentage of the total soluble matter (%TSM) was calculated using the following equation:

$$\text{TSM(\%)} = \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100 \quad (2)$$

TSM tests for each type of film were performed in three replicates.

2.3.8. Water vapor transmission rate (WVTR)

The water vapor transmission rate was determined gravimetrically using a modified ASTM method E96–95. Plastic cups, with an average diameter of 2 cm and a depth of 4.5 cm were utilized to determine the WVTR of the films. Initially, films were cut into discs with a diameter slightly larger than the diameter of the cup. Approximately 3.0 g of anhydrous CaSO_4 was placed in each cup and sealed with transparent films of varying composition using epoxy glue to maintain the RH at 0% in the cups. All cups were placed in a desiccator. Use of a saturated solution of K_2SO_4 (25 °C) created a RH of 97 % in the desiccator. Cups were weighed every 2 h for a day then every 12 h for two days and then measurements were carried out every 24 h for 2 days. The amount of water that permeated through the film was determined based on the weight that was gained in the cups throughout the time frame of measurements. Tests were conducted in duplicate for each sample and water vapor transmission rate ($\text{g Pa}^{-1} \text{ h}^{-1} \text{ m}^{-1}$) was calculated.

2.3.9. Oxygen transmission rate (OTR)

OTR of nanocomposite films was analyzed on a MOCON OX-TRAN[®] 2/21 (Modern Controls Inc., Minneapolis, MN, USA) apparatus equipped with a coulometric oxygen sensor. Samples were placed in a specimen area of 5 cm² aluminum masks and sealed with epoxy. The ASTM standards that were used include D–3985 and F–1927. The film was exposed to 100% oxygen on one side and a mixture of 98% nitrogen and 2% hydrogen on the other side. Oxygen permeability (OP) values were calculated by dividing the oxygen transmission rates by the differential partial pressure of oxygen

across the film (1 atm or 101.3 kPa) and multiplying by the film thickness (Nisperos-Carriedo, Krochta & Baldwin, 1994). The measurements were carried out in duplicate at 23 °C, 50% RH for 24 h. The OP was reported as cc mil (m⁻²day⁻¹atm⁻¹), whereas the OTR was reported as cc (m⁻²day⁻¹).

$$OP = \frac{OTR \cdot l}{\Delta p} \quad (3)$$

where l is the sample thickness and Δp is the difference in partial pressure of oxygen at the two sides of the films. Δp was 1 atm in this study.

2.3.10. Thermal gravimetric analysis (TGA)

Thermogravimetric analyses of different films were carried out in duplicate, using a Thermo Scientific SDT Q600 series Thermogravimetric Analyzer (TA instrument). Samples weighed between 9–11 mg and were heated from room temperature to 700 °C in alumina cups at a heating rate of 10 °C/min. All experiments were performed under N₂ at a flow rate of 35 mL/min in order to prevent degradation by thermal oxidation.

2.3.11. Mechanical properties

The mechanical properties of the conditioned films were measured with an Instron model 3345 testing machine with a 50 N load cell. All tests were carried out according to ASTM D882–12 standards and at least five specimens were tested for each composition. Films were cut into 70 mm ×10 mm rectangles and conditioned at 50% relative humidity and 23 °C for at least 5 days prior to analysis. Each film specimen was mounted between the grips (lined with rubber). The initial separation distance between the grips was set at 50 mm and the cross-head speed was 5 mm/min, respectively. Tensile stress (TS, MPa), young's modulus (Y, MPa) and elongation at break (ϵ_b , mm) were calculated using Bluehill 2 software.

3. Results and Discussion

3.1. *Film thickness and conditioning*

In order to enhance film properties, it is necessary to introduce a second component into the chitosan polymer. The drying step involved in film formation causes contraction in film to some extent after evaporating the solvent. An addition of TEMPO-CNFs has improved the film formation and prevented the contractions during drying process as well. TEMPO-CNFs allow chitosan to form continuous films of various shapes easily without any deformation (Nordqvist, Idermark, Hedenqvist, Gällstedt, Ankerfors & Lindström, 2007). The films made of different compositions of TEMPO-CNFs were homogeneous, flexible, and transparent. Film thickness was dependent on the film nature and composition (Table 1). Films containing higher amounts of TEMPO-CNFs were slightly thicker than the 100% chitosan film. This thickness change may be caused by the compact differences between chitosan chains, anions of TEMPO-CNFs and their interactions. It is known that chitosan and CNFs are miscible polymers via hydrogen bonding interactions. Both polymers contain free –OH groups. CNFs are also soluble in water due to their hydrophilic nature. Interestingly, TEMPO-oxidized CNFs exhibit free anionic carboxylate groups on their surfaces, which interact with the free ammonium cations of chitosan molecules. When TEMPO-CNFs are added to the chitosan matrix, an interconnected bond network can be formed through electrostatic interactions and hydrogen bonding. Also, the one component film of chitosan/CNFs ratio 100/0 dried more quickly than 80/20 and 75/25 two component films. This suggests that unbound water molecules were trapped within the network of the two component films and therefore, evaporated more slowly (Hartman, Albertsson, Lindblad & Sjöberg, 2006). The sorbitol has a low molecular weight and therefore may intercalate between both

polymeric chains, spacing them a part, and thus make the film softer. Therefore, a self-supporting, flexible and continuous film can be obtained by incorporating TEMPO-CNFs into chitosan matrix.

Table 1

Development of bionanocomposite films of various compositions based on chitosan/TEMPO-CNFs.

Samples (wt/wt)	Chitosan (wt%)	TEMPO CNFs (wt%)	Sorbitol (wt%)	2% Acetic acid solution (g/mL)	Casting solution (mL)	Thickness (mm)	Moisture content (wt%)
100/0	100	0	25	1.25/150	100	0.1143	6.7
95/5	95	5	25	1.25/150	100	0.1219	7.5
90/10	90	10	25	1.25/150	100	0.1498	8.1
85/15	85	15	25	1.25/150	100	0.1295	7.8
80/20	80	20	25	1.25/150	100	0.1346	8.4
75/25	75	25	25	1.25/150	100	0.1498	10.2

3.2. Field emission scanning electron microscopy

SEM images of the bionanocomposite films and fractured surfaces by tensile testing are shown in Fig. 1. The surface morphology showed the proper dispersion and tightly packed structure of TEMPO-CNFs into chitosan matrix. Fig. 1a showed a uniform morphology with small flakes or dots that tend to accumulate on the film surface. All nanocomposite films with uniform thickness were smooth, without any macroscopic bubbles, cracks, and pores. Surfaces of film composition 100/0 and 95/5 were smooth and flat whereas films surface with different compositions (90/10, 85/15, 80/20, and 75/25) exhibited a homogeneous, but wavy surface. Higher TEMPO-CNFs containing films were little rough because of very small needle shaped (Soni, Hassan & Mahmoud, 2015) TEMPO-oxidized nanofibers. There was no aggregation of TEMPO-CNFs

observed that indicates, TEMPO-CNFs were dispersed homogeneously with chitosan matrix, thus formed strong interaction and adhesion on the interfaces of both polymeric chains. This excellent dispersion of nano-sized TEMPO-CNFs in the blend matrix was directly correlated with its effectiveness in improving the properties of bionanocomposite films.

The fractured surfaces of nanocomposite films in Fig. 1b, explained the influence of TEMPO-CNFs onto chitosan matrix. Bionanocomposite film compositions of 95/5 to 75/25 displayed that TEMPO-CNFs appear to be embedded in the chitosan matrix. They have dense structure due to rigid hydrogen bonded network and due to their higher crystallinity. Moreover, an increase of surface roughness is evident. The SEM micrographs also provided the evidence of the good dispersion of TEMPO-CNFs on the chitosan matrix without noticeable aggregation even for films with high reinforcement ratios (85/15, 80/20, and 75/25).

Fig. 1. FE-SEM micrographs of different chitosan/TEMPO-CNFs bionanocomposite films, (Fig.1a) surface morphology and (Fig.1b) fractured surface morphology

3.3. Atomic force microscopy

The topography of produced films is analyzed by Atomic Force Microscopy. All images with higher resolution were recorded to determine the surface structural information, whereas the height images were generated for the additional morphology and surface roughness of the films. Observed AFM images are shown in Fig. 2. Height images revealed that the needle shaped, very small sized TEMPO-CNFs with diameter of 3–20 nm and length 10–100 nm (Soni, Hassan & Mahmoud, 2015) were strongly connected to chitosan polymer and formed composed structure. Due to high aspect ratio and in-build structure in chitosan matrix, it was difficult to identify TEMPO-CNFs, even if their content was very high (20–25 wt %). The surface of films 80/20 and 75/25 showed rough orientation, similar morphology was reported for the other chitosan based films (Fernandes et al., 2009) as well as films of other nanocomposites such as, nanocellulosic xylan (Saxena, Elder, Pan & Ragauskas, 2009), and polyvinyl alcohol-cellulose (Cheng, Wang & Rials, 2009). In this study, TEMPO-CNFs were in contact with each other and surrounded by chitosan molecules that leads to a continuous network between both of them, thus filler was evenly distributed within the polymeric matrix.

Fig. 2. AFM images of different chitosan/TEMPO-CNFs bionanocomposite films.

3.4. X-ray diffraction

The X-ray diffractograms of all film compositions displayed similar diffraction peaks (Fig. 3). A sharp distinct peak was observed at ($2\theta = \sim 44.6^\circ$) indicating that the films produced with 2% acetic acid casting method are mainly crystalline. 100% chitosan films showed two major broad peaks at 2θ of approximately 9.77° and 19.88° . Addition of TEMPO-CNFs slightly displaced the position of these peaks to higher angles and increased their intensity. The differences in peak intensity and displacement between 100% chitosan film and chitosan/TEMPO-CNF films were not significant. This indicating that the crystallinity of the prepared films slightly increased with increasing the content of TEMPO-CNF. This phenomenon is most likely explained by the organized deposition

of chitosan chains on the surface of the crystalline domains of the TEMPO-CNFs. The presence of sorbitol does not change the crystalline structure. Similar results have been reported for chitosan based composite films with nanofibrillated cellulose, bacterial cellulose, and sulfonated cellulose (Fernandes et al., 2010; Fernandes et al., 2009).

Fig. 3. X-ray diffraction of different chitosan/TEMPO-CNFs bionanocomposite films.

3.5. Optical Transmittance

The UV-vis spectra of all films are presented in Fig. 4. In the range of 200-800 nm, 100% chitosan film showed optical transmittance of 85.9% whereas 95/5, 90/10, and 85/15 films have 77.5%, 69.1% and 62.4% transmittance. Films 80/20 and 75/25 showed less optical transmittance (58.1% and 51.2%) which indicated that addition of higher amounts of TEMPO-CNFs (20-25 wt%) decreases the transparency of film.

Fig. 4. Optical transmittance of different chitosan/TEMPO-CNFs bionanocomposite films.

3.6. Moisture absorption of the films

Incorporation of various amounts of TEMPO-CNFs in the chitosan matrix reduced water absorption in the films (Fig. 5). Hydrogen bonds and electrostatic interactions between the negatively charged carboxylate groups on the TEMPO-CNFs surface and the ammonium groups of chitosan were the driving forces for such improvement in the

water resistibility (de Mesquita, Donnici & Pereira, 2010; Krumova, López, Benavente, Mijangos & Pereña, 2000). The high number of hydroxyl groups (–OH) in the 100/0 chitosan/CNFs film absorbed 12.6% moisture. A gradual decrease in total water absorption was achieved as CNF inclusion concentration increased from 95/5 to 75/25. Moisture absorption was stabilized for all films by 24 h due to saturation. Similar results were obtained by other researchers who observed a reduction of water absorption when a composite was made with the incorporation of hydroxypropylmethylcellulose (Bilbao-Sáinz, Avena-Bustillos, Wood, Williams & McHugh, 2010; Sebti, Chollet, Degraeve, Noel & Peyrol, 2007), wood hydroxylate (Edlund, Ryberg & Albertsson, 2010), and spruce galactomannan (Mikkonen, Heikkilä, Helén, Hyvönen & Tenkanen, 2010) into different natural biopolymers. The moisture uptake decreased as the TEMPO-CNFs content increased.

Fig. 5. Moisture absorption of different chitosan/TEMPO-CNFs bionanocomposite films.

3.7. Barrier properties of the films

Incorporation of TEMPO-CNFs into the chitosan matrix was able to increase the water solubility of films. Water solubility increased from 12.8% to 51.9% as TEMPO-CNFs content was increased from 0 to 25 wt % (Fig. 6a). Carbonyl groups in cellulose nanofibers can form strong electrostatic interactions with ammonium groups on chitosan and thus decrease water sensitivity. However, increasing TEMPO-CNFs concentration increased the water solubility of the films. This can be explained as a result of the chitosan and TEMPO-CNFs having a greater affinity for water than each other since ammonium and carboxylate groups can interact more easily with water molecules and form a strong hydrogen bonding network. This reduces the cohesiveness between film components and therefore increases the water solubility of the films. Accordingly, chitosan films with high TEMPO-CNF are not desirable for preserving liquid food or liquid-containing food. Adding hydrophobic agents during manufacturing of chitosan/TEMPO-CNF films might be necessary to decrease water solubility.

Water vapor transmission rate (WVPR) is the most important feature of bionanocomposite films for their application in food industry. The mechanism of water vapor transmission (WVTR) is a diffusion process in which water vapor condenses and dissolves on the film surface and then liquid water diffuses through the film. Bionanocomposite films must prevent moisture transfer between food and the surrounding atmosphere to be used in food packaging applications. The WVTR of 100% chitosan films was $3.28 \times 10^{-8} \text{ g Pa}^{-1} \text{ h}^{-1} \text{ m}^{-1}$ (Fig. 6a). Increasing the concentration of cellulose nanofibers caused a gradual decrease in the WVTR of the films. The film compositions of 95/5, 90/10 and 85/15 lead smaller WVTR values ($3.26 \times 10^{-8} \text{ g Pa}^{-1}$

427 $\text{h}^{-1}\text{m}^{-1}$, $3.23 \times 10^{-8} \text{ g Pa}^{-1} \text{ h}^{-1}\text{m}^{-1}$, and $3.22 \times 10^{-8} \text{ g Pa}^{-1} \text{ h}^{-1}\text{m}^{-1}$) than 100% chitosan
428 films. This could be attributed to the hydrophilic $-\text{OH}$ groups in the of chitosan polymer
429 not interacting with the TEMPO-CNFs because the concentration is too low (5–15 wt
430 %). Furthermore, in films with low TEMPO-CNFs concentration (100/0, 95/5, and
431 90/10), chitosan might conglomerate, which would negate the functionality of the TEMPO-
432 CNFs and thus facilitate water vapor permeation through the matrix. However, WVTR
433 values ($2.89 \times 10^{-8} \text{ g Pa}^{-1} \text{ h}^{-1}\text{m}^{-1}$ and $2.63 \times 10^{-8} \text{ g Pa}^{-1} \text{ h}^{-1}\text{m}^{-1}$) of films with 80/20 and
434 75/25 chitosan/CNFs ratio were less than the others. It explains that increment of
435 TEMPO-CNFs into chitosan matrix increases the inter chain between both the polymers
436 which leads to reduce water vapor diffusivity through the film and obstruct the water
437 vapor transmission. This also agree with results reported for other packaging
438 applications (Famá, Gerschenson & Goyanes, 2009; Ma, Chang & Yu, 2008; Soni,
439 Hassan & Mahmoud, 2015). This corresponds to the highly crystalline and hydrophobic
440 character of the TEMPO-CNFs that disperse well in the chitosan matrix and block the
441 water vapor transmission (Fujisawa, Okita, Fukuzumi, Saito & Isogai, 2011). Also, as
442 reported previously, the sorption and the diffusion phenomena took place exclusively in
443 the amorphous phase of the polymer and not in its highly crystalline zones. The
444 crystalline portion increases the effective path length of gas diffusion and seems to
445 reduce the mobility of the polymer chains because chain-ends are blocked in the
446 crystalline phase lamellae (Pauly, 1989). Therefore, the decrease in water vapor and
447 oxygen permeability for films with 80/20 and 75/25 chitosan/CNFs ratio is another
448 evidence for the crystallinity increase which was observed by XRD.

Oxygen transmission rate (OTR) measures the amount of gas that passes through a substance over a given period of time. Oxygen transmission can lead to oxidation, which triggers several food changes such as odor, color, flavor, and nutrient deterioration. Accordingly, obtaining nanocomposite films with proper oxygen barrier can help improving food quality and extending food self-life. OTR and oxygen permeability (OP) values of chitosan based nanocomposite films with various loads of TEMPO-CNFs are shown in Fig. 6b. TEMPO-CNFs based nanocomposite films had a greater barrier to oxygen than the film made of 100% chitosan. Nanocomposite films with 80/20 and 75/25 compositions showed excellent oxygen barrier properties. In the previous study, SEM and AFM analysis showed that TEMPO-oxidized CNFs have a road like structure with a width of 3–20 nm and length of 10–100 nm (Soni, Hassan & Mahmoud, 2015). Therefore, TEMPO-CNFs form a compact structure with chitosan molecules due to strong intermolecular attraction, which prevents gas from permeating through the films.

A high oxygen barrier film has an oxygen transmission rate range of approximately 1–10 cc ($\text{m}^{-2}.\text{day}^{-1}$) (Abdellatief & Welt, 2013). The average oxygen transmission rate for 100% chitosan film was 0.45 cc ($\text{m}^{-2}.\text{day}^{-1}$) which was less than nanocellulose (0.74 cc ($\text{m}^{-2}.\text{day}^{-1}$)) (Sirviö, Kolehmainen, Visanko, Liimatainen, Niinimäki & Hormi, 2014), xylan (354.950 cc ($\text{m}^{-2}.\text{day}^{-1}$)), and 25% sulfonated nanocrystalline cellulose/xylan (1.038 cc ($\text{m}^{-2}.\text{day}^{-1}$)) film (Saxena, Elder, Kenvin & Ragauskas, 2010), but greater than the films with TEMPO-CNFs reinforced into the chitosan matrix (0.21 to <0.05 cc ($\text{m}^{-2}.\text{day}^{-1}$)). OTR values of packaging films prepared from commercial polyethylene terephthalate (PET), polyamide-ethylene vinyl alcohol (PA-EVOH-PA), and

polypropylene-ethylene vinyl alcohol (PP-EVOH-PP) have been reported in the range of 110, 0.5, and 0.3 cc (m⁻².day⁻¹), respectively (Brody, Bugusu, Han, Sand & McHugh, 2008; Mogna, 2014; Strupinsky & Brody, 1998). Therefore, the OTR values of TEMPO-CNFs blended chitosan films in this study were comparable with OTR values of packaging films that are currently utilized in the food industry. These results indicate that chitosan/TEMPO-CNFs nanocomposite films can be used as a natural packaging to protect food from oxidation reactions.

Fig. 6. Water and oxygen barrier properties of different chitosan/TEMPO-CNFs bionanocomposite films.

3.8. Thermal gravimetric analysis

Fig. 7 shows that all films decreased in weight at 100 °C due to the evaporation of absorbed and intermolecular H-bonded water and acetic acid (Kumar, Negi, Choudhary & Bhardwaj, 2014; Li, Wang & Liu, 2011; Soni, Hassan & Mahmoud, 2015). The amount of absorbed water in 100/0 film is higher than that of the 75/25 film, suggesting that the TEMPO-CNFs were well dispersed within the chitosan matrix, thus preventing the absorption of moisture by the films. The TGA curve represented by weight (%) and solid lines, thermal degradation of the 100/0 chitosan film and other TEMPO-CNFs reinforced films initiated at 240–250 °C under N₂ (Fig. 7). In DTG curve presented by deriv. weight (%/°C) and dashed lines, all films showed prominent pyrolysis with single step degradation and the major associated weight loss was observed from 220–380 °C. In our previous study, thermal degradation of TEMPO-CNFs alone was initiated at 215 °C and entire pyrolysis temperature range was 180–350 °C (Soni, Hassan & Mahmoud, 2015). This decrease in their degradation temperature was due to their nano-size and larger number of free ends. In this study, there are no much difference in the degradation temperature of all nanocomposite films, these films showed higher thermal stability, which is mainly due to the presence of the crystalline structure and great compactness between both chitosan and TEMPO-CNFs. This result revealed that the introduced –COOH groups can influence the thermal stability of the chitosan based composites.

Fig. 7. TGA-DTG thermograph of different chitosan/TEMPO-CNFs bionanocomposite films.

3.9. Mechanical properties of the films

The average values of tensile stress, tensile strain, young's modulus and elongation at break of the prepared films are presented in Fig. 8. In general, tensile strength increases and the strain at break decreases as the amount of cross linking agent increases. In a similar way, the stress and strain mechanical properties curve (Fig. 8a) of nanocomposite films showed the transition from ductile to plastic behavior when the TEMPO-CNFs concentration was increased from 5 to 25 wt %. 100% chitosan films were relatively weak as indicated by the maximum stress of 10.7 MPa. The addition of higher TEMPO-CNFs content (20–25 wt %) increased both tensile stress and young's modulus from 17.5–18.7 MPa and 545–652 MPa, which were much higher than the 100% chitosan films. This indicates a high load bearing capacity. Nanocomposite films

80/20 and 75/25 showed a much higher young's modulus and more rigid feature that can be attributed due to the formation of a TEMPO-CNFs and chitosan network. The graphs of young's modulus (Y) and elongation at break (ϵ_b) (Fig. 8b) indicate that the highest Y and Lowest ϵ_b values were associated with 75/25 films. This could be attributed to the cross linking effect, strong electrostatic interactions and hydrogen bonds of TEMPO-oxidized nanofibers in the chitosan matrix. Similar results were reported for other natural macromolecule based films reinforced with CNFs. For example, the tensile stress of xylan rich hemicellulose films increased from 11.9 to 39.5 MPa, and young's modulus increased from 735 to 3304 MPa by incorporation of 20 wt % CNFs into the film (Peng, Ren, Zhong & Sun, 2011). Likewise, the tensile stress of an amylopectin film increased from 0.35 to 15.0 MPa, and young's modulus increased from 1.6 to 780 MPa by incorporation of 20 wt % CNFs into the film (Svagan, Azizi Samir & Berglund, 2007). Improvement of tensile strength as a consequence of addition of TEMPO-oxidized CNFs could be attributed to the high aspect ratio, good mechanical strength of TEMPO-CNFs and the strong interaction between carboxylate groups of TEMPO-CNFs and the ammonium groups of the chitosan molecule. This interaction between nanocomposite film components could restrict the segmental mobility of the polymer chains in the vicinity of the nanoreinforcement and the interface destroying (Siqueira, Bras & Dufresne, 2008). These results show the possibility of forming chitosan/TEMPO-CNFs based films with high mechanical performance. Good mechanical cohesion was formed, which was a prerequisite for the evolution of material properties.

Fig. 8. Mechanical properties of different chitosan/TEMPO-CNFs bionanocomposite films.

Elongation at the break is the percentage change in the original film length between the grips, the final length is measured when the film breaks. It was interesting that the extension at break did not reduce to zero with the increase of the TEMPO-CNFs contents from 5 to 25 wt %. It was hypothesized that TEMPO-CNFs could improve the film strength without impacting flexibility. The lowering of elongation at break is a common trend that is affected by the volume fraction of the added reinforcement, the dispersion in the matrix, and the interaction between the

reinforcement and the matrix (Colom, Carrasco, Pages & Canavate, 2003; Fortunati et al., 2012). These results further indicate that TEMPO-CNFs can be used for nanoreinforcement and to improve the mechanical properties of the chitosan films.

4. Conclusions

TEMPO-oxidized cellulose nanofibers were introduced into a chitosan matrix using an environmentally friendly casting method to prepare biodegradable nanocomposite films.

Higher crystallinity of TEMPO-CNFs and the dense composite structure of the produced films leads to a film that has reduced moisture and oxygen transmission properties. The produced nanocomposite films possessed higher thermal stability, mechanical properties, transparency and flexibility when compared to 100% chitosan films. SEM and AFM images indicated that nano-sized TEMPO-CNFs were completely embedded in the chitosan matrix. Results from this study indicated that incorporation of TEMPO-CNFs into a chitosan matrix was a feasible, green, and effective method for application in transparent biodegradable packaging that can be used for food products.

Acknowledgment

This research was funded by the USDA-ARS Agreement 58-6402-2-729, and the CRIS project number MIS-501170.

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