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Development, characterization, and validation of chitosan adsorbed cellulose nanofiber (CNF) films as water resistant and antibacterial food contact packaging

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ABSTRACT

Compatibility of CNF with three polysaccharides having different surface charges and backbones (chitosan, methyl cellulose, and carboxymethyl cellulose) was investigated. Chitosan (CH) incorporation reduced water absorption (WA) of CNF films ($P < 0.05$). CH molecular weight (Mw) (68, 181, 287 kDa) and amount (10 and 20 g/100 g CNF in dry basis) impacted moisture barrier, mechanical, antibacterial, thermal, and structural properties of CNF films. Regardless of Mw, CH incorporation (20 g/100 g CNF) decreased ($P < 0.05$) WA of CNF films, and high Mw (287 kDa) CH (20 g/100 g CNF) incorporation resulted in lower film water solubility while increasing film water vapor permeability compared with low Mw CH (68 kDa) incorporation ($P < 0.05$). CNF film with low Mw CH (20 g/100 g CNF) exhibited antibacterial activity against *L. innocua* and *E. coli*. Interaction mechanisms between CH and CNF were investigated through thermal, structural, and morphology analyses using DSC, FTIR, and SEM, respectively. CNF films with low or high Mw CH incorporation (20 g/100 g CNF) were further validated as surface contact films for fresh beef patties, showing effectiveness to prevent moisture transfer between the layered patties. This study demonstrated the potential of using CNF-CH composite films as water resistant and antibacterial packaging for foods with high moisture surfaces.

1. Introduction

Cellulose nanofiber (CNF) forms films with superior mechanical and gas barrier properties because of its nano-sized dimension, high aspect ratio, surface area, and flexibility (Azeredo, 2009; H.P.S. et al., 2016). However, due to the hydrophilic surface property of CNF, those functional properties of the CNF films may be suppressed by direct moisture contact and/or exposure to high relative humidity (RH) environment (Liu, Walther, Ikkala, Belova, & Berglund, 2011; McHugh, Avena-Bustillos, & Krochta, 1993), thus limiting their application for packaging food products with wet surface, high moisture content, and/or stored at high RH environment. Incorporation of inorganic fillers (i.e. silver), chemical modification (i.e. plasma polymerization or derivatives), and adsorption of other film matrix materials (i.e. xyloglucan or guar gum) have been studied to enhance the water-resistance of CNF films (Eronen, Junka, Laine, & Österberg, 2011; Hernández-Hernández, Neira-Velázquez, Ramos-de, Ponce, & Weinkauf, 2010; Lavoine, Desloges, & Bras, 2014). Among these methods, the adsorption of polymeric materials onto CNF surface avoids the use of strong or harmful chemicals, and is simple, safe and efficient, hence was investigated in this study to develop water resistant packaging films for food with wet and adhesive surfaces.

Polysaccharides are classified based on their surface charges and backbones. Both carboxymethyl cellulose (CMC) and methyl cellulose (MC) are composed of glucose monomers, but contain negatively-charged carboxymethyl (-CH_2COOH) and non-charged relatively hydrophobic methoxyl (-OCH_3) functional groups, respectively. Chitosan (CH) is composed of β-(1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine with positively-charged amino (-NH_2) groups in acidic solution. The chemical interactions (e.g. hydrogen bonds or electrostatic interactions) and/or physical interactions (e.g. adsorption) of these polysaccharides onto CNF surface vary depending on their functional groups, surface charges, molecular weights (Mw), concentrations, and conformation (Lin & Dufresne, 2014). This study thus selected three types of polysaccharides (CH, MC, and CMC) for understanding their affinities onto CNF surface with different surface charges and backbones and

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possible impact on the film water-resistant property.

Our previous studies proved that the abundance and availability of the functional amino groups and spatial entanglement of CH vary depending on molecular weight (Mw) of CH (Chen & Zhao, 2012; Jung & Zhao, 2012, 2013). Hence, CH incorporation at different Mw and concentrations into CNF film was tested in this study based on the hypothesis that the affinity of CH with CNF depends on Mw and concentration of CH due to different abundance and availability of the functional amino groups, spatial entanglement and crystallinity of CH, which in turn could impact the physicochemical, mechanical, and antibacterial properties of CNF films. The derived CNF-based films were expected to have sufficient water-resistant and antibacterial properties that can be applied as food contact packaging film to interleave food products with high moist (e.g. meat pieces or patties) surface, thus preventing moisture transfer between layered products.

Therefore, the objectives of this study were to first identify the mostly compatible polysaccharide with CNF to develop water-resistant CNF films and then to validate their applications for contacting food items with wet surfaces and stored under high RH refrigerated temperature. The former objective was achieved through combined two experimental designs: (1) Taguchi design to select the mostly compatible polysaccharide (CMC, MC, or CH) with CNF and (2) completely randomized two factorial design to investigate the influences of Mw and concentration of CH on the properties of CNF films. The derived films were evaluated in physicochemical (color, thickness, haze), water-resistant (water absorption, water solubility, water vapor permeability), mechanical (tensile strength, elongation at break), thermal (differential scanning calorimetry (DSC)), structural (Fourier transform infrared spectroscopy (FT-IR)), and morphological (scanning electron microscopy (SEM)) properties, as well as antibacterial activity against *Listeria innocua* (*L. innocua*) and *Escherichia coli* (*E. coli*). The latter objective was accomplished by applying CNF films with the best performance based upon the first part of studies to layered beef patties as the separation sheets, and evaluating water absorption of films after 1-week refrigerated storage. This study was thus anticipated to provide new insights on the strategies of incorporating functional polysaccharides into CNF for enhancing water resistance and antibacterial activity of CNF films to meet the critical needs of biodegradable antibacterial packaging films and for understanding the mechanisms of improved performance of CNF film based on Mw and concentration of incorporated CH.

2. Materials and methods

2.1. Materials

A CNF slurry (2.95 g/100 g wet basis) was obtained from the Process Development Center of the University of Maine (ME, USA). CNF was extracted from northern bleached softwood kraft pulp by using the Masuko MKZB15-50J super mass collider creating a high shear zone, thus liberating nanofibers present in natural lignocellulosic fibers with dimensions of 20–50 nm in width and up to several hundred microns in length (The Process Development Center, University of Maine, 2016). CMC (400–800 cPs), MC (400 cPs), and CH (97% degree of deacetylation, 287 kDa Mw) were purchased from Alfa Aesar (MA, USA), Sigma Chemical (MO, USA), and Premix (Iceland), respectively. Glycerol was acquired from Fisher Scientific (NJ, USA). Tween 80 and Span 80 were obtained from Amresco (OH, USA). Cellulase was gained from *Aspergillus niger* (TCI America, OR, USA). Ground beef (80% lean and 20% fat) was purchased from a local market on the day that experiment was conducted.

2.2. Chitosan depolymerization

CH was depolymerized to different Mw levels (high: 287 ± 43 kDa, med: 181 ± 18 kDa, and low: 68 ± 2 kDa) through enzymatic hydrolysis using the method from our previous study (Jung & Zhao, 2013). Briefly, high Mw CH (287 kDa) (1 g/100 g) was dissolved in acetic acid (1 g/100 mL distilled water) and adjusted to pH 5 using 10 g/100 mL NaOH. Cellulase (10 g/100 g CH in dry basis) was added to prepared chitosan solutions, and reacted at 50 °C for 5 min or 1.5 h to obtain med or low Mw CH, respectively. The hydrolyzates were boiled for 10 min to inactivate cellulase, and centrifuged at 8500×*g* for 30 min to remove denatured enzyme. Supernatant was then adjusted to pH 9 by NaOH (10 g/100 g in distilled water), and the precipitated portion was washed and collected by centrifugation at 8500×*g* for 30 min. Collected samples were dried at a 40 °C oven overnight. The viscosity-average Mw of CH (0.01 g/100 g 0.1 M CH_3COOH and 0.2 M NaCl) was measured by the Ubbelohde dilution viscometer (Cannon Instrument Co., PA, USA) with a capillary size of 0.58 nm. The intrinsic viscosity was determined by the intercept between the Huggins (reduced viscosity) and Kraemer (relative viscosity) plots. The viscosity-average Mw of CH was calculated using Mark–Houwink–Sakurada (MHS) equation: $[\eta] = K (M_w)^a$, where $K = 1.81 \times 10^{-3}$ mL g^{-1}, a = 0.93, and $[\eta]$ represented the intrinsic viscosity (Jung & Zhao, 2013).

2.3. Preparation of CNF-based films

2.3.1. Development of film formulations

Film formulations were developed using two consecutive experimental designs, Taguchi design (the first part of study) and a completely randomized two factorial design (the second part of study). For Taguchi design (Table 1), CNF (0.75 g/100 g distilled water) and glycerol (10 g/100 g CNF in dry basis) were formulated with different types (CMC, MC, and CH) and concentrations (0, 15, and 30 g/100 g CNF in dry basis) of polysaccharides, avocado oil (0, 15, and 30 g/100 g CNF in dry basis), and surfactant mixture (1:1 of Tween and Span 80, 0, 20, and 40 g/100 CNF in dry basis). Mw of CH used for this part of the study was 287 kDa. Avocado oil was chosen to increase the hydrophobicity of the films, and surfactant was selected to improve the incorporation of hydrophobic compound and decrease the surface tension of the derived films. For a completely randomized two factorial design (Table 2), CNF (0.5 g/100 g distilled water) for creating thinner film and glycerol (10 g/100 g CNF in dry basis) were used along with the addition of the mostly compatible polysaccharide identified from the Taguchi design. In this study, CH was identified as the mostly effective polysaccharide, and different Mw (68, 181, and 287 kDa) and concentrations (10 and 20 g/100 g CNF in dry basis) of CH were incorporated into CNF film formulations. For each concentration of CH, prepared CH solution (3 g/100 mL acetic acid (1 g/100 g distilled water)) was diluted 60 and 30 times, respectively. In this case, the concentration of acetic acid was <0.05 g/100 g for both concentrations of CH. It was previously reported that antibacterial activity of acetic acid starts at 0.166 g/100 mL (Fraise, Wilkinson, Bradley, Oppenheim, & Moiemen, 2013). Hence, the influence of acetate on antibacterial activity of films was negligible in this study.

2.3.2. Preparation of films

Prepared film formulations were homogenized (Polytron PT10-35, Luzernerstrasse, Switzerland) for 5 min, and degassed using a self-build water flow vacuum system (Chen & Zhao, 2012). A 60 mL of formulation was uniformly cast onto 150 mm diameter polystyrene petri dish (Falcon, PA, USA), and dried at room conditions (20 ± 2 °C and 30± 2% RH) for 2 days. Dried films were then conditioned in a self-assembled chamber (Versa, PA, USA) at 25 °C