Food Packaging and Shelf Life 17 (2018) 73–79

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

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

Physicochemical, antimicrobial and antioxidant properties of chitosan/TEMPO biocomposite packaging films

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ARTICLE INFO

Keywords:
Chitosan
Cellulose nanofibers
Thermal stability
Bacteria
Packaging materials

ABSTRACT

Chitosan-based biocomposite films have attracted considerable attention due to their versatile physicochemical properties, and higher antimicrobial and antioxidant activities. This study investigated the physiochemical properties and examined the antimicrobial and antioxidant activities of chitosan/TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical) bio-composite films. The films were prepared by incorporating different ratios of chitosan (100, 85, and 75 wt.%), TEMPO cellulose nanofibers (0, 15, and 25 wt.%) and sorbitol (25 wt.%). The bio-composite films were casted in an oven at 40 °C for 2–4 days. The successful incorporation of chitosan was confirmed by Fourier transform Infrared spectroscopy (FTIR) and thermal stability (TGA) measurements. The antimicrobial activity results showed a significant reduction in the growth of *Salmonella enterica, E. coli O157:H7,* and *Listeria monocytogenes* bacteria on the surface of the films with the proportional increase of chitosan. Results also showed a significant increase in the antioxidant activity of films with high chitosan concentration. The improved antimicrobial and antioxidant activities indicate that such films can be used successfully as packaging materials for several foods.

1. Introduction

Nowadays, packaging material is one of the major challenges that modern food industries are facing. High-quality food without chemical preservatives is the prime demand of consumers. Food packaging material with antimicrobial and antioxidant properties is an emerging technology that could have a significant impact on shelf life extension and food safety. The antimicrobial and antioxidant compounds present in packaging material can serve as a carrier to keep high concentrations of preservatives on the food surfaces. Therefore, the preparation of antimicrobial packaging films with the use of natural resources is an area of increasing interest being explored (No, Meyers, Prinyawiwatkul, & Xu, 2007; Reesha, Panda, Bindu, & Varghese, 2015; Soni, Hassan, Schilling, & Mahmoud, 2016). Biobased packaging materials could improve food quality and enhance the shelf life of food products; they are intended to function as barriers against oxygen, aroma, moisture, flavor, and oil (Rhim, Hong, Park, & Ng, 2006; Wong, Camirand, & Pavlath, 1994). In addition, biopolymer based films are excellent mediums for incorporating a broad collection of additives such as antimicrobials, antioxidants, antifungal agents, colors, and other nutrients (Han, 2003; Park & Zhao, 2004). Particularly, biopolymer based antimicrobial and antioxidant packaging films are gaining much attention from food industries for their potential applications in a variety of products including poultry, meat, cereals, cheese, fruits, and vegetables (Cha & Chinnan, 2004; Han, 2003; Shiekh, Malik, Al-Thabaiti, & Shiekh, 2013).

Chitosan 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). Development of active biodegradable packaging films in order to improve the nutritional stability, quality and extend the storage life of food is a potential utilization of chitosan biopolymer (Chen, Zheng, Wang, Lee, & Park, 2002). Chitosan possesses strong antimicrobial, antioxidant and antifungal activities as reported by several researchers (Dutta, Tripathi, Mehrotra, & Dutta, 2009). In spite of these unique properties and numerous advantages of chitosan, its films exhibit poor mechanical functionalities as well as a weak barrier against water vapor and gases, which limits its unexclusive applications in food packaging fields. Therefore, multiple approaches have been applied to improve the barrier and mechanical performance of chitosan based films (Soni et al., 2016). In recent years, a substantial amount of research that deals with the blending of chitosan with various natural biopolymers, such as

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https://doi.org/10.1016/j.fpsl.2018.06.001
Received 5 February 2018; Received in revised form 2 May 2018; Accepted 5 June 2018

starch (Bourtoom & Chinnan, 2008), cellulose (Shih, Shieh, & Twu, 2009; Velásquez-Cock et al., 2014), and several other cellulosic derivatives (Abou-Zeid et al., 2011; Dayarian, Zamani, Moheb, & Masoomi, 2014; Li, Chen, & Wang, 2015) have been reported. Polymer blending is the most efficient strategy to formulate biopolymer based films with desired properties (Soni et al., 2016).

The formation of biopolymer based composite films from the utilization of green polymers is becoming an increasing acknowledged alternative for future material production for a more sustainable society. Particularly, chitosan and cellulose are primary interests due to their structural similarity which can result in materials that merge the physicochemical properties of chitosan with impressive mechanical characteristics of cellulosic fibers (Wu et al., 2004; Yin, Luo, Chen, & Khutoryanskiy, 2006). Cellulose is the most common polysaccharide on earth and a classic example of a renewable resource, which can be used to generate potential reinforcing biomaterials called cellulose nanofibers. In the last several decades, the advancement of cellulose nanofibers (CNFs) has gained considerable attention due to their low cost, low density, renewability, and nonabrasive nature. All these important characteristics of CNFs allow them to create bio-composites with easy process ability and make them attractive candidates in the nanomaterial research fields. In our previous study, transparent bio-nanocomposite films based on chitosan and TEMPO-oxidized cellulose nanofibers with enhanced mechanical and barrier properties were successfully prepared (Soni et al., 2016). These completely individualized cellulose nanofibers (CNFs) have been extracted from cotton stalks (Soni, Hassan, & Mahmoud, 2015) by TEMPO-mediated oxidation (2,2,6,6-tetramethylpiperidine-1-oxyl radical) under moderate aqueous condition (Saito, Kimura, Nishiyama, & Isogai, 2007). This oxidation phenomenon coupled with ultrasonication process selectively converts primary alcohols (−OH) to aldehyde (−CHO) and finally carboxylate groups (−COO) (Soni et al., 2015, 2016). The width and length of TEMPO-CNFs was in range from 3–15 nm and 10–100 nm.

This study aims to further examine the physicochemical characteristics of chitosan/TEMPO-CNFs films, and to examine their antimicrobial and antioxidant properties. The antimicrobial activity of these films was demonstrated against a Gram-positive bacterium *(Listeria. monocytogenes)* and a Gram-negative bacterium *(Escherichia coli and Salmonella. enterica)*. The antioxidant activity was determined using DPPH and ABTS scavenging analytical assays.

2. Material and methods

2.1. Materials

Cellulose was isolated from cotton stalks by alkaline-acid pretreatment and used for the preparation of nanocellulose by the TEMPO-mediated oxidation method (Soni et al., 2015). Chitosan (DD ~72%) was synthesized from Mississippi gulf brown shrimp *(*penaeus aztucus*)* exoskeletons. All chemicals used in this study were purchased from commercial resources and used as received without any further purification. The following chemicals were purchased from Fisher Scientific, USA. Sorbitol, glacial acetic acid, ethanol (95%), hydrochloric acid, sodium hydroxide, potassium hydroxide, potassium persulfate, acetic acid, and TEMPO [(2,2,6,6-tetramethylpiperidin-1-yl) oxy radical]. ABTS [2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)], DPPH (2,2-Diphenyl-1-picrylhydrazyl), and Trolox were purchased from sigma Aldrich, USA. Teflon petri dish (140 mm × 140 mm) liner was purchased from Fluoro Lab, USA. Bacterial strains (ATCC), yeast extract and peptone water from Becton Dickinson, MD, USA.

2.2. Preparation and characterization of chitosan

Shrimp exoskeletons were obtained from Mississippi Gulf Coast, Biloxi, MS, which were dried in the oven at 40 °C for 48 h, grinded and screened through universal vibrator screen into 30 mesh − 4 mm particle size and finally the moisture content was determined. The grinded shrimp exoskeletons were placed in Ziploc bags and refrigerated. Chitin was separated to several treatment steps for preparation of chitosan. In the first step, the grounded shells were soaked in 4% HCl solution (1:14 w/v) at 23 °C for 40 h to remove minerals (mainly $CaCO_3$), then the remaining chitin was filtered and washed with distilled water till neutrality. In the second step, the demineralized chitin was treated with 5% NaOH solution (1:12 w/v) at 90 °C for 24 h to eliminate proteins and sugars from crude chitin. After cooling, chitin was collected and washed with distilled water. In the third step, chitin was deacetylated by 70% NaOH solution (1:14 w/v) at 23 °C for 75 h to produce chitosan, which was washed several times with distilled water to obtain neutral pH, followed by filtration to yield creamy white product. Finally, the moisture content was determined and the dried chitosan was kept in Ziploc bags for characterization.

2.3. Characterization of chitosan

2.3.1. Moisture content determination

Moisture content of prepared chitosan was performed according to NREL/TP-510-42621 method. The water mass was determined by drying the sample to constant weight and measuring the sample after and before drying. The water weight was the difference between the weights of the wet and oven dry samples.

2.3.2. Ash content

To determine the ash content value of chitosan, 2.0 g of chitosan sample was placed into previously ignited, cooled, and tarred crucible. The samples were heated in muffle furnace preheated to 700 °C for 4 h. the crucible was allowed to cool in the furnace to less than 200 °C and then placed into desiccator. Ash content was determined according to NREL/TP-510-42622 procedure. Percentage of ash value is calculated using the following equation:

2.3.3. Degree of deacetylation (%) by potentiometric titration

Chitosan (0.25 g) was dissolved in 30 mL of 0.1 M HCl and diluted with 10 mL of deionized water. Under continuous stirring, 0.1 M NaOH was added dropwise until the pH reached a value of 3. A value of f(x) of the corresponding volume of NaOH added was calculated using the following formula:

$$f(x) = \left(\frac{V_0 + V}{N_B}\right) \times ([H^+] - [OH^-]) \quad (1)$$

Where V_0 is the volume of chitosan solution (mL), V is the volume of NaOH added (mL), N_B is the concentration of NaOH (M), $[H^+]$ is the concentration of H^+ (M), $[OH^-]$ is the concentration of OH^- (M). A linear titration curve was obtained by plotting f(x) vs. corresponding volume of NaOH. By extrapolating the linear titration curve to the x-axis, the volume of NaOH at the end point can be estimated. Five replicates were performed for synthesized chitosan sample. The degree of deacetylation (DD) was calculated using the following formula (Tan, Khor, Tan, & Wong, 1998)

$$DD\ (\%) = \frac{\phi}{\frac{(W - 161 \times \phi)}{204} + \phi} \times 100 \quad (2)$$

$$\phi = \frac{(N_A \times V_A - N_B \times V_B)}{1000} \quad (3)$$

where N_A is the concentration of HCl (M), V_A is the volume of HCl (mL), N_B is the concentration of NaOH (M), V_B is the volume of NaOH at the end point (mL), and W is the sample mass (g).

2.3.4. Degree of deacetylation (%) by elemental analysis

The degree of deacetylation value of chitosan samples was calculated from the following formula (Jiang, Chen, & Zhong, 2003; Kasaai, Arul, & Charlet, 2000):