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Thermal and antimicrobial properties of chitosan–nanocellulose films for extending shelf life of ground meat

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ABSTRACT

Chitosan–nanocellulose biocomposites were prepared from chitosan having molecular weight of 600–800 kDa, nanocellulose with 20–50 nm diameters and various levels of 30, 60 and 90% (v/w_{CHT}) for glycerol. Agitation and sonication were used to facilitate even dispersion of particles in the polymer matrix. The nanocomposites were examined by differential scanning calorimetry, X-ray diffraction and agar disc diffusion tests; finally, the film was applied on the surface of ground meat to evaluate its performance in real terms. Chitosan–nanocellulose nanocomposites showed high T_g range of 115–124 °C and were able to keep their solid state until the temperature (T_m) range of 97–99 °C. XRD photographs revealed that nanocellulose peak completely disappeared after their addition to chitosan context. Agar disc diffusion method proved that the nancomposite had inhibitory effects against both gram-positive (*S. aureus*) and gram-negative (*E. coli* and *S. enteritidis*) bacteria through its contact area. Application of chitosan–nanocellulose nanocomposite on the ground meat decreased lactic acid bacteria population compared with nylon packaged samples up to 1.3 and 3.1 logarithmic cycles at 3 and 25 °C after 6 days of storage, respectively.

1. Introduction

An area of growing interest, these days, is the preparation of antimicrobial edible films applied for controlling foodborne microbial outbreaks mainly caused by minimally processed fresh products (Güçbilmez, Yemenicioğlu, & Arslanoğlu, 2007). Chitosan (CHT), the cationic (1-4)-2-amino-2-deoxy-β-D-glucan, is industrially produced in various quality grades from chitin, the second most abundant polysaccharide in nature (Muzzarelli, 2012; Tome et al., 2013; Muzzarelli et al., 2012). Chitin and chitosan are natural antimicrobial compounds against an extensive variety of microorganisms including bacteria, yeasts and moulds (Vu, Hollingsworth, Leroux, Salmieri, & Lacroix, 2011). Chitosan is a non-toxic and biodegradable compound and has excellent performance in forming films (Mayachiew, Devahastin, Mackey, & Niranjan, 2010). Chitosan films have successfully been used as packaging materials for the preservation of food quality (Fernandes et al., 2009), which motivated us to select this carbohydrate polymer as a matrix context for preparing desirable edible films.

Cellulose consists of D-glucopyranose units joined together by β-(1→4)-glycosidic bonds and could be found in wood, cotton, hemp, etc. (Khan et al., 2012). Cellulose nanocrystals have low densities, high elongation moduli and tensile strengths (Dadashi, 2011; Klemm, Heublein, & Fink, 2005); besides, they have high biodegradability rates and are less expensive than other nanofillers (Hansson et al., 2013). Nanocellulose (n-cellulose) particles are very suitable nanomaterials for the production of cheap, lightweight, and very strong nanocomposites; meanwhile, they are more effective than their microsized counterparts to reinforce polymers (Azeredo et al., 2010).

Nanocomposites (NCPs) are novel polymer matrices which have been incorporated by nanoparticles having at least one dimension in nanoscale (Petersson & Oksman, 2006). At the same time, chitosan–cellulose compounds are of particular interest because of the structural similarity between these two biopolymers (Fernandes et al., 2009). Khan et al. (2012) incorporated 1–10% (w/w) n-cellulose particles with 5–10 nm width into chitosan and analyzed mechanical and barrier properties of prepared

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NCPs. De Mesquita, Donnici, Teixeira, & Pereira (2012) incorporated n-cellulose particles having 145 nm length and 6 nm diameter into chitosan matrix and studied structural characteristics of obtained films. Fernandes et al. (2010) prepared chitosan–nanocellulose NCPs from low and high molecular weight chitosan powders (and their modified ones) and 5–60% (w/w) nanocellulose; they investigated mechanical and thermal properties of prepared nanocomposites and reported that the addition of NCL increased degradation temperatures of chitosan films up to 45 °C. However, there are not any researchers dealing with thermal (compared with synthetic polymers) and antimicrobial properties of this NCP and this has led to some confusion about practical applications of the nanocomposites for industrial uses, having been evaluated in the current research.

Microbial growth commonly imposes undesirable organoleptic changes during meat storage; therefore, preventing the bacterial growth at interfacial level of meat surface-packaging film is of paramount importance to both producers and consumers groups of these products. Kim et al. (2011) reported that chitosan films with high viscosity (100 and 200 mPa s) did not exhibit any antimicrobial effect against *Escherichia coli* and *Salmonella typhimurium*; although, those were effective against *Listeria monocytogenes*. No, Park, Lee, and Meyers (2002) reported that the growth of *E. coli* was inhibited more efficiently by chitosan having high molecular weight compared with low molecular weight ones. The aim of this research was to prepare nanocomposites deserving favourable thermal properties (compared with synthetic polymers) for the food industry and to display antimicrobial advantages of chitosan–nanocellulose biocomposites, which could be regarded as their superiority over common synthetic polymers. Also, our objective was to evaluate efficiency of chitosan–nanocellulose biocomposites in a real food model system for the first time in order to reveal any possible significant improvements in the product's shelf life as it is in a great demand for the meat industry nowadays.

2. Materials and methods

2.1. Materials

Chitosan powder, having molecular weight of 600–800 kDa, and cellulose nanoparticles, with 20–50 nm diameters, were purchased from Acros Organics Co., Belgium and Asahi Kasei Corp., Japan, respectively. Pure acetic acid and glycerol were obtained from Merck Co., Germany. Culture media of Nutrient Broth, Brain Heart Infusion (BHI), de Man Rogosa Sharpe (MRS) Broth and Agar and Bacto-Peptone were purchased from Merck Co., Germany and Hi Media Co., India, respectively. *E. coli* PTCC 1399, *Staphylococcus aureus* PTCC 1431 and *Salmonella enteritidis* PTCC 1381 were supplied by Persian Type Culture Collection, Iran.

2.2. Film preparation

Certain amounts of chitosan powder (Table 1) were dissolved in 40 mL aqueous solution (1% v/v) of glacial acetic acid; stirring was conducted at 50 °C and 250 rpm by a heater-stirrer (CB162, Stuart®, UK) for 2 h. In parallel, certain amounts of n-cellulose (Table 1) were added to 20 mL distilled water and dispersed in the same conditions as chitosan dissolution for 2 h. Finally, the latter solution was added to the former one. Glycerol (Table 1) was dispersed in the solution and thorough homogenization occurred in the similar temperature and speed to chitosan dissolution for 24 h. Then, the solution was homogenized with a rotor stator (IKA®T25 digital, Ultra-Turrax®, Germany) at 8000 rpm for 15 min and an ultrasound device (TI-H-10, Elma®, Germany) at 35 kHz and 100% power without heating for 30 min. Deaeration was conducted in a thermostat vacuum oven (Croydon, Townson & Mercer Ltd., UK) at 600 mmHg for 1 h without heating. The solutions (60 mL) were casted on the centre of glass plates, having 100 cm^2 surface areas and placing inside the oven at 37 °C; the required time for film forming was 48 h. The dried films were removed from the plates and placed in the oven for two further days to evaporate residual solvents completely and finally were located in hermetic packaging plastics ($18 \times 14\ cm^2$; Pollyetilen Co., Iran).

Final film (after overall optimization) was prepared from 1% (w/0.6v) chitosan powder, 0.18% (w/w_{CHT}) n-cellulose and 30% (v/w_{CHT}) glycerol to apply on the surface of ground meat (Dehnad, Emam-Djomeh, Mirzaei, Jafari, & Dadashi, 2014).

2.3. Thermal analysis

Thermal properties of the NCPs were studied by Differential Scanning Calorimetry (DSC 1, Mettle Toledo, Swiss). 10 mg sample was placed in aluminium DSC pans; the device was calibrated against indium as standard. For the measurements, 4 specimens including sample Nos. 1, 3, 5 and 14 were evaluated. Each sample heated by the instrument under an argon atmosphere at 20 mL/min velocity and heating rate of 10 °C/min up to 170 °C, followed by cooling to −10 °C and reheating to 200 °C (Cerqueira, Souza, Teixeira, & Vicente, 2012). Characteristics of melting peaks and glass transition temperatures (T_g) were determined on the basis of first and second scans, respectively (Mucha & Pawlak, 2005).

2.4. X-ray diffraction

Samples were analyzed between $2\theta = 5–45°$ with step increment of $2\theta = 0.02°$ in a Bruker Diffractometer (D8 Advance, Siemens, Germany) using a Cu Kα irradiation (40 kV/30 mA) at the wavelength of 1.54 Å (Li et al., 2011). Sample Nos. 2, 4, 6 and 8 were selected to study the effect of different n-cellulose and glycerol levels on microcrystalline structure of NCPs.

2.5. Agar disc diffusion test

E. coli, *S. aureus* and *S. enteritidis* were grown on the nutrient agar slant and kept at 4 °C. To prepare liquid culture of bacteria, a whole loop of each bacterium was cultured into 50 mL BHI sterile medium. Then, cultures of bacterial strains (in BHI) were agitated at 140–150 rpm and 37 °C for 24 h in a shaker incubator (SI50, Stuart®, UK). A dilution series was carried out to meet required bacterial population for seeding, by using sterile distilled water. Agar diffusion method was used for determining antibacterial properties. Films were cut into squares having 10 or 15 mm sides (with special moulds) for *E. coli* and *S. aureus* or *S. enteritidis*, respectively. Film pieces were placed on BHI Agar. Agar plates had been previously seeded with 0.1 mL of an overnight broth culture for indicator strains. Initial number of bacteria was in the range of 10^5–10^6 CFU/mL. Bacterial strains were incubated at 37 °C for 24 h (Pranoto, Rakshit, & Solakhe, 2005). Results were reported as ability (−) or lack of ability (+) for bacterial growth in the contact area with nanocomposites.

2.6. Shelf life investigation of ground meat

Efficacy of the optimized antimicrobial films was evaluated by placing films on the upper surface of 60-mm diameter circular slabs (15 mm thickness) of a ground meat sample (100 g) which was purchased from a local market in Gorgan, Iran. The slabs were aseptically prepared and placed in the bottom sections of petri dishes and then completely covered with chitosan–nanocelullose or nylon films. The sets were individually thermo-sealed in oxygen impermeable films ($13 \times 13\ cm^2$; PakizehPeyk Plastic Industry,