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Physicochemical and antifungal properties of bio-nanocomposite film based on gelatin-chitin nanoparticles

Samar Sahraee[a], Jafar M. Milani[a], Babak Ghanbarzadeh[b,*], Hamed Hamishehkar[c]

[a] Department of Food Science and Technology, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran
[b] Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, P.O. Box 51666-16471, Tabriz, Iran
[c] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

The gelatin-based nanocomposite films containing chitin nanoparticles (N-chitin) with concentrations of 0, 3, 5 and 10% were prepared and their physical, thermal and anti-microbial properties were investigated. Scanning electron microscopy (SEM) micrographs showed that N-chitin size distribution was around 60–70 nm which dispersed appropriately at low concentration in gelatin matrix. The results showed that incorporation of N-chitin significantly influenced apparent color and transparency of the gelatin films. The reduced water vapor permeability (WVP) and solubility and higher surface hydrophobicity of the nanocomposite films were obtained by enhancing N-chitin concentration in film formulation. The use of N-chitin up to 5% concentration in the gelatin based nanocomposite film led to improved mechanical properties. Also, the results of differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) confirmed improved stability of nanocomposite films against melting and degradation at high temperatures in comparison to neat gelatin film. The well compatibility of chitin nanoparticles with gelatin polymer was concluded from Fourier transform infrared (FTIR) spectra and X-ray diffraction (XRD) plots. Finally, the gelatin based nanocomposite films had anti-fungal properties against *Aspergillus niger* in the contact surface zone. Increasing the concentration of N-chitin up to 5% enlarged inhibition zone diameter, but the nanocomposite film containing 10% N-chitin showed smaller inhibition zone.

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1. Introduction

Nowadays, the tendency to use biodegradable packaging in food products is increasing due to enhanced concerns about extra environmental pollution resulted from non-degradable polymers as wastes [1]. In this regard, two types of biodegradable polymers have been developed: edible and non-edible biopolymers. Carbohydrates and proteins or the combination of them usually are used as source of edible biopolymers [2].

One of the most important protein sources of biopolymers is gelatin which is formed by chemical denaturation of collagen gained from skin, bone or tissues of animals and generally is considered for its appropriate rheological film forming properties [3]. However, the lack of desired mechanical, thermal and water vapor barrier properties has led to impossibility of using this material alone in food packaging.

One of the relatively new solutions for improving biodegradable materials in order to create sufficient properties as a packaging material is incorporation of nanoparticles into film formulation and enhancing packaging properties such as barrier, mechanical and thermal behavior [4,5]. Therefore, different varieties of nanoparticles such as metal oxides, nanoclays, crystalline nano whiskers or nano fibers have been introduced to polymers' formulation. So far, investigations on nanoparticles applied for gelatin polymers such as AgNPs and nanoclay/gelatin [6], montmorillonite/gelatin [7], N-ZnO/gelatin [4], TiO_2/gelatin [8], N-chitosan/gelatin [9] nanocomposites were reported. Meanwhile, organic nanoparticles have some advantages comparing to inorganic ones such as usually coming from food processing byproducts, being compatible to biodegradable polymers, being edible, nontoxic and non dose-limited [10]. In this case, chitin nano fibers have attracted much attention because of their availability and antimicrobial properties.

Chitin is one of the most abundant polysaccharides in the nature which can be achieved from fungi wall, stiff shell of marine animals, insects and etc. with the chemical structure of β-(1–4)-linked 2-

* Corresponding author.
E-mail addresses: Samar.sahraee@gmail.com (S. Sahraee), Jmilany@yahoo.com (J.M. Milani), Ghanbarzadeh@tabrizu.ac.ir (B. Ghanbarzadeh), Hamishehkar.hamed@gmail.com (H. Hamishehkar).

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acetamido-2-deoxy-D-glucopyranose units [10,11]. In recent years, different methods of preparing chitin whiskers applied as filler in composite films have been reported [12–14]. In all methods, it has been attempted to remove the amorphous phase and gain rigid high crystalline fibers which can be embedded in protein matrix as nanofiller [15]. Over the last few years, some studies have detected nano chitin (N-chitin) influence on mechanical, thermal and barrier properties of biopolymers. For example, Wang et al. [16] observed improved properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) composite film containing N-chitin. Also, Incorporation of chitin nanoparticles in starch [15], carrageenan [10], chitosan [11] and soy protein [13] matrix in order to promote polymer capabilities for food packaging have been studied as well.

On the one hand, chitin, chitosan and their derivatives have active structures with functional groups i.e. amino groups which are compatible with protein structures like gelatin. On the other hand, both chitin and gelatin are obtained from the most available sources in the nature, making them interesting for industries. Therefore, in this study, it has been attempted to investigate the effects of N-chitin as nanofiller on physico-mechanical properties of gelatin-based composite films as a material for food packaging.

2. Materials and methods

2.1. Materials

Bovine gelatin was supplied by Merck Chemical Co. (Darmstadt, Germany), with bloom number of 200 and density of 1358 kg/m^3. Liquid glycerol and 50% glutaraldehyde were provided by Sigma Chemical Co. (St. Louis, MO, USA). Nano-chitin gel was bought from Nano-shimi Yakhte Co. (Mazandaran, Iran) with 1.5% dry matter and particle size of 50–70 nm. In order to study antifungal activity, *Aspergillus niger* strain was obtained from (Microbial collection of Drug Applied Research Center, Tabriz University of medical science) and grown on SDS Agar slants at 25 °C.

2.2. Preparation of gelatin/nano chitin nanocomposite films

N-Chitin concentration of 3, 5 and 10% wt on dry gelatin basis was homogenized in 100 ml distilled water with a magnetic stirrer for one hour. Then, it was transferred to an ultrasound bath (Parsonic 30S, Pars Nahand engineering Co. Iran) and held for further homogenization. After that, 4 g/100 ml gelatin was added to this solution and held for 30 min at 7 °C to be swelled [17]. After heating this solution at 55 °C for 30 min, glycerol (30% wt. on dry gelatin) was added as a plasticizer and stirred for 30 min at 35 °C. Then, 50%wt Glutaraldehyde aqueous solution (1%wt on dry gelatin basis) was added during stirring at 35 °C to the dispersion as a crosslinking agent and was stirred for 30 min further. All of the films were prepared by casting 100 ml of this solution on Teflon-coated plates (diameter 16 cm) and drying in ambient temperature for 48 h. Conditioning of the films was done by keeping them in 55 ± 2% RH for 48 h before experiments. Control films were prepared as described for nanocomposite films without adding N-chitin to the solution [18].

2.3. Characterization of nanocomposite films

2.3.1. Microstructural studies by scanning electron microscope (SEM)

Determination of the size of chitin nanoparticles and surface morphology of gelatin/N-chitin nanocomposite films was done by SEM (Mira3 TE, Czech Republic). In order to determine the particle size of N-chitin, chitin gels were dissolved in distilled water by ultrasonic treatment and after drying for 48 h at room temperature, transferred to SEM specimen holder to be scanned at accelerating voltage of 10.0 kV [6]. Moreover, nanocomposite films were held on SEM specimen and analyzed at 26.0 kV voltage and current of 10.0 μA.

2.3.2. Solubility

In order to determine the solubility of films, three pieces of each sample (2 × 2 cm^2) were weighed after drying at 105 °C in oven for 24 h and immersed in 100 ml distilled water at 25 °C for 24 h. Then, the samples were filtered via a filter paper and dried at 105 °C for 24 h to determine the amount of residual dry mater. Solubility of film was calculated as follow:

$$\% S = \frac{(m_i - m_d)}{m_i} \times 100 \quad (1)$$

Where S is solubility of films, m_i expresses the initial weight of the dried film and m_d remarks the weight of dried residual films after immersion [19].

2.3.3. Water vapor permeability (WVP)

The WVP of films were determined by modification of ASTM E96-95 standard method [20]. Glass vials with average depth of 4 cm, diameter of 1.5 cm and perforated doors with average hole diameter of 4 mm were used to measure WVP. The films were cut into sphere shapes with diameters of 1 cm and placed on the top of vial which contained 3 g dried $CaSO_4$ salt to create an environment with 0% RH and stiffened by vial door. The entire vial was weighed and transferred to a desiccator with 97% RH at 25 °C. The weight of each vial was measured at 24 h intervals during 7 days. The slop of the linear part of the curve was determined as water vapor transmission rate (WVTR) and WVP was calculated as follow:

$$WVP = (WVTR \times L)/\Delta P \quad (2)$$

Where L is the average thickness of the film and ΔP is the difference of partial water vapor pressure.

2.3.4. Surface hydrophobicity of the films

To compare the hydrophobicity of the nanocomposite films surface, the water drop contact angle (CA) on the surface of each film was determined according to the method introduced by Liu et al. [21]. Rectangular pieces of the films (2 cm × 5 cm) were placed on the black box (10 cm × 10 cm) and a black background was fixed behind the surface of the box to inhibit light reflex when taking photos. Then 10 μl drop of water was carefully put on the films' surfaces from 1 cm height. Subsequently images of both sides of the drop were recorded by digital camera (Canon, IXY, 12.1 megapixels)., contact angles were measured by Adobe Acrobat 9.0 professional and results were stated as an average of three measurements and results were presented as the average amount of three measurements.

2.3.5. Mechanical properties

To study the effect of nanoparticles on mechanical properties of gelatin-based films, ultimate tensile strength (UTS), Young's modulus (EM) and elongation at break (EB) were measured using a Microelectronics Universal Testing Instrument Model DBBP-20 (Bongshin, Korea). The cross-head speed was 10 mm/min at 25 °C and the initial grip separation was set at 50 mm. All of the samples were cut into dumbbell-shaped pieces with 10 cm length and 1 cm width, and were equilibrated in 55 ± 2% RH.

2.3.6. Color and transparency

To describe the color of the films, a hunter lab instrument was used. A white color plate was used as background and each sample

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