International Journal of Biological Macromolecules 84 (2016) 153–160

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Fabrication of gelatin–TiO_2 nanocomposite film and its structural, antibacterial and physical properties

Qingyan He, Yuchen Zhang, Xixi Cai, Shaoyun Wang*

College of Biological Science and Technology, Fuzhou University, Fuzhou 350108, China

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form
30 November 2015
Accepted 9 December 2015
Available online 12 December 2015

Keywords:
Nanocomposite film
Structural
Antibacterial

ABSTRACT

Biodegradable fish skin gelatin–titanium dioxide (TiO_2) nanocomposite films were fabricated and characterized as a function of incorporating amount of TiO_2 nanoparticles (gelatin/TiO_2 ratio of 30:1, 20:1 and 10:1). A uniform distribution of TiO_2 nanoparticles into gelatin matrix was observed using atomic force microscopy (AFM) micrographs. The data of intrinsic fluorescence spectra, Fourier transform infrared spectra (FTIR) and X-ray diffraction confirmed the interaction between protein and nanoparticles through hydrogen bonding. The TiO_2-incorporated gelatin nanocomposite films exhibited more effective antibacterial activity for *Escherichia coli* after irradiating 120 min by UV light (365 nm), which were 54.38% for *E. coli* and 44.89% for Staphylococcus aureus, respectively. The analysis of physical properties revealed that addition of TiO_2 nanoparticles to gelatin films significantly increased the tensile strength and elongation at break, while decreased its water vapor permeability. The light barrier measurements indicated that these films were highly transparent, and they had excellent barrier properties against UVC light at the same time. The results demonstrated the feasibility of incorporating nanoparticles to improve the properties of gelatin films, and it is of significance in utilizing the gelatin and titanium dioxide to produce biodegradable nanocomposite film as packaging material in food industry.

1. Introduction

The edible biodegradable films based on naturally renewable biopolymers have attracted much attention in past decades, which are primarily composed of polysaccharides, proteins and lipids [1]. Several studies have been conducted on protein-based packaging materials originated from plant and animal such as soy isolate protein, zein, gluten, whey protein and gelatin [2–4].

Gelatin is a denatured fibrous protein, extracted from skins, bones and tissue of animals. Due to religious reasons and the occurrence of bovine spongiform encephalopathy, gelatin obtained from skin, bone and scale of seafood processing by-products has gained great interest [5,6]. Edible gelatin films prepared from fish skin had better barriers against oxygen. However, the low mechanical and high water solubility of gelatin films did have limitations in their application.

Titanium dioxide (TiO_2) nanoparticles, an inert, nontoxic oxide, have been investigated via combining it with metals to extend its absorption edge into the visible region. For example, Sui et al. reported that the photocatalytic of the Ag–AgBr/TiO_2 heterostructured nanofibers is far superior to that of TiO_2 nanofibers and Ag–AgBr power [7]. Zhao et al. found that the formation of oxygen vacancies in TiO_2 was promoted by the metallic Cu nanoparticles, which results in the enhancement of visible-light absorption of Cu@TiO_2 [8]. However, TiO_2 nanoparticles have been approved for using in food color additive [9]. Edible films based on organic polymer incorporated with nanoparticles could improve the mechanical, thermal or barrier properties when compared with the pure polymers [10–12]. Moreover, due to its photocatalytic actives, TiO_2 nanoparticles as potential antibacterial agents can be used to provide the protection against foodborne microorganisms in the presence of ultraviolet radiation [13]. The presence of nano-TiO_2 particles might induce the conformational transition of silk fibroin to Silk II structure and improve the mechanical and thermal properties of silk fibroin/nano-TiO_2 nanocomposite films [14]. The association of whey protein–TiO_2 was reinforced when at a low TiO_2 concentration, while self-assembly of TiO_2–TiO_2 would enhance at a high TiO_2 concentration [15]. The antibacterial and physical properties of polyethylene-based film incorporated with TiO_2 nanoparticles were investigated by Xing et al. [16]. Zolfi et al. [13] had characterized the uniform distribution of TiO_2 in kefiran–whey protein isolate film by scanning electron microscopy.

However, there is little information available on the effects of TiO_2 nanoparticles on the properties of biodegradable films pre-

* Corresponding author. Fax: +86 591 22866278.
E-mail address: shywang@fzu.edu.cn (S. Wang).

http://dx.doi.org/10.1016/j.ijbiomac.2015.12.012
0141-8130/

pared from fish skin gelatin. In order to improve the properties and expand the antibacterial functions of gelatin films, the objective of this work was therefore to evaluate the effect of TiO_2 nanoparticles addition in fish skin gelatin film on its structural, physical properties and antibacterial activity.

2. Materials and methods

2.1. Materials and chemicals

Shark skin was purchased from the local market of Fuzhou, China. Nano-titanium dioxide (TiO_2) solution was obtained from the State Key Laboratory Breeding Base of Photocatalysis, Fujian, China. *Escherichia coli* (*E. coli*) ATCC 25922 and *Staphylococcus aureus* (*S. aureus*) ATCC 29213 were provided by the Fujian Academy of Agricultural Sciences. All the other chemicals (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Fuzhou, China).

2.2. Extraction of shark skin gelatin

Gelatin was extracted from shark skins according to the method of Nagarajan et al. [17] with a slight modification. The treated skins were cut into small pieces and then soaked in 0.1 M NaOH (1:6, w/v) at 4 °C overnight with stirring. Then the skins were washed with water to neutral pH and drained. After the alkaline treatments, the skins were soaked in 0.05 M CH_3COOH (1:6, w/v) at 4 °C for 4 h with a continuous stirring and then were washed until a neutral pH of wash water was obtained and drained. The swollen skins were soaked in 6 times of distilled water (w/v) at 70 °C for 6 h to extract gelatin. After centrifuging at 12,000 × *g* for 20 min, the obtained supernatant was condensed 8 times under vacuum at 50 °C. Protein concentration of concentrated solution was determined by Lowry's method.

2.3. Preparation of TiO_2/gelatin nanocomposite film

Gelatin solutions (15 mg/mL) and glycerol (40% of protein, w/w) were magnetically stirred to obtain uniform solutions. After that, the pH of solution was adjusted to pH 4.0 with 0.1 M NaOH. Then the solution heated at 40 °C for 2 h in a water bath and cooled at room temperature, followed by vacuum degassing to remove dissolved air bubbles. TiO_2 nanoparticles were added slowly with gently stirring at 30:1, 20:1, 10:1 (gelatin:TiO_2, w/w) to obtain the film-forming solution. Solutions were cast onto a rimmed polypropylene plate (9 × 9 cm^2) and dried at 25 ± 0.5 °C and 50 ± 5% relative humidity (RH) for 24 h. Dried film samples were peeled off and stored at 25 ± 0.5 °C and 50 ± 5% (RH) for subjecting to further study.

2.4. Structural characterization of TiO_2/gelatin nanocomposite film

2.4.1. Size and zeta-potential

The particle size and zeta potential in TiO_2–gelatin nanocomposite film forming solutions were measured using a Malvern Laser Scattering Particle Analyzer (Malvern Instruments Ltd., Malvern, Worcestershire, UK) at 25 °C.

2.4.2. Intrinsic fluorescence spectroscopy

The fluorescence spectra of the gelatin and TiO_2–gelatin nanocomposite films were recorded using a 970CRT fluorescence spectrometer (Precision Instruments Co. Ltd., Shanghai, China). The measurements were performed using an excitation wavelength of 280 nm and emission spectra from 290 to 500 nm. Excitation and emission slits were set at 10 nm.

2.4.3. X-ray diffraction

The crystal structure of the pure TiO_2 nanoparticles, gelatin and TiO_2–gelatin nanocomposite films were obtained using an X' Pert Pro MPD diffractometer (Phillips Co., Holland). All samples were exposed to a nickel-filtered Cu Kα radiation beam at a voltage of 40 kV and current of 30 mA with 2θ range of 5°–80°.

2.4.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of gelatin and TiO_2–gelatin nanocomposite films were recorded using FTIR spectroscopy (Thermo Nicolet Co., USA) on reflection mode from 4000 to 400 cm^{-1}. For each spectrum, 64 scans at a resolution of 4 cm^{-1} were obtained. The peak signals in the spectra were analyzed using OMNIC 8.2.

2.4.5. Atomic force microscopy (AFM)

The surface of gelatin and TiO_2–gelatin nanocomposite film were measured by using atomic force microscopy (AFM) on a TM–AFM 5500 (Agilent Technologies, USA) in tapping mode at constant forces. After the samples were diluted to the concentration of 0.01 mg/mL, 5 μL of the solution was dropped on a freshly cleaved mica surface. Then it was spread and dried under a stream of nitrogen.

2.5. Antibacterial properties of TiO_2/gelatin nanocomposite film

The antibacterial properties of nanocomposite film against the bacteria *E. coli* and *S. aureus* were studied according to Wang et al. [4]. Single colony of fresh bacteria was scraped and incubated at 37 °C for 20 h. The bacterial suspensions were diluted into 10^5 CFU/mL of *E. coli* and 10^4 CFU/mL of *S. aureus*, respectively. Two groups of films were investigated, one group contained TiO_2 nanoparticles and the other one without TiO_2 as a blank. The films were placed into sterilized Petri dishes and then 1 mL of bacteria suspension was added onto the surface of each film. The samples were irradiated to an ultraviolet lamp at 365 nm for 120 min at room temperature. Then the samples were washed with 0.85% NaCl solution in order to remove the adhered bacteria in the sterilized Petri dishes. 100 μL of bacteria suspension was cultured on the agar medium and incubated for 24 h at 37 °C. The antibacterial activity (R) was calculated by the following Eq. (1):

$$R(\%) = \left(1 - \frac{E}{B}\right) \times 100 \quad (1)$$

where E is the viable bacterial number of experimental group, B is the viable bacterial number of blank group.

2.6. Physical properties of TiO_2/gelatin nanocomposite film

2.6.1. Film thickness

The thickness of gelatin films and TiO_2–gelatin nanocomposite films were measured using a micrometer caliper (Measuring & Cutting Tools Co. Ltd., Shanghai, China). Seven measurements were taken at random locations.

2.6.2. Mechanical properties

Tensile strength (TS) and elongation at break (EBA) were determined by using a Texture Analyzer (TA series, Stable Micro System Co. Ltd., UK) according to the described by Hoque et al. [18] with slight modification. The film samples were cut into rectangles strips (20 × 50 mm^2), clamped on the grips of the device with an initial grip of 30 mm, and deformed at crosshead speed of 1 mm/s. TS (MPa) and EBA (%) were calculated by the following Eqs. (2) and (3):

$$TS\,(MPa) = \frac{F_m}{(L \times W)} \quad (2)$$