International Journal of Biological Macromolecules 80 (2015) 460–468

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Preparation and application of agar/alginate/collagen ternary blend functional food packaging films

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ARTICLE INFO

Article history:
Received 6 April 2015
Received in revised form 6 July 2015
Accepted 8 July 2015
Available online 15 July 2015

Keywords:
Silver nanoparticles
Grapefruit seed extract
Antimicrobial food packaging

ABSTRACT

Ternary blend agar/alginate/collagen (A/A/C) hydrogel films with silver nanoparticles (AgNPs) and grapefruit seed extract (GSE) were prepared. Their performance properties, transparency, tensile strength (TS), water vapor permeability (WVP), water contact angle (CA), water swelling ratio (SR), water solubility (WS), and antimicrobial activity were determined. The A/A/C film was highly transparent, and both AgNPs and GSE incorporated blend films (A/A/C^{AgNPs} and A/A/C^{GSE}) exhibited UV-screening effect, especially, the A/A/C^{GSE} film had high UV-screening effect without sacrificing the transmittance. In addition, the A/A/C blend films formed efficient hydrogel film with the water holding capacity of 23.6 times of their weight. Both A/A/C^{AgNPs} and A/A/C^{GSE} composite films exhibited strong antimicrobial activity against both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli*) food-borne pathogenic bacteria. The test results of fresh potatoes packaging revealed that all the A/A/C ternary blend films prevented forming of condensed water on the packaged film surface, both A/A/C^{AgNPs} and A/A/C^{GSE} composite films prevented greening of potatoes during storage. The results indicate that the ternary blend hydrogel films incorporated with AgNPs or GSE can be used not only as antifogging packaging films for highly respiring fresh agriculture produce, but also as an active food packaging system utilizing their strong antimicrobial activity.

1. Introduction

General concerns on the environmental impact such as exhaust of natural resources, energy crisis, global warming, and ecological problems caused by the petroleum-based plastic packaging materials has aroused interests in the development of biodegradable packaging materials using biopolymer-based materials [1,2]. Ever since various types of biopolymers and biopolymer-based packaging systems have been developed as an alternative for the non-biodegradable plastic packaging materials [3]. As one of such biopolymer-based packaging materials, biopolymer-based hydrogel films have been introduced for the packaging of fresh fruit and vegetable with high respiration rate [4]. Hydrogel films are tridimensional polymer networks which possess the ability to absorb and retain large amounts of water when placed in water or biological fluids without dissolution. Especially, biopolymer-based hydrogels gained particular interests in medical, pharmaceutical, and food packaging industries due to their abundant availability, sustainability, biodegradability, and biocompatibility [4–6]. However, poor mechanical and water resistance properties of most biopolymer-based materials limit their industrial use. Variety of efforts have been made to overcome such problems including physical, chemical, and enzymatic treatments or blending with hydrophobic additives or other polymers with different structures. Generally, composite formation through blending with nanofillers such as nano-sized clay, metal or metallic oxides, and crystalline cellulose fibers, polymer–polymer combination, and chemical cross linking methods have been suggested as useful methods for improving or modifying the physiochemical properties of biopolymer-based hydrogel films [7].

One of the most widely studied approaches is to form nanocomposite by incorporating silver nanoparticles (AgNPs) into biopolymer films [8–12]. This is mainly on account of strong and broad-spectrum of antimicrobial activity of AgNPs against bacteria, viruses, and fungi. In addition, mechanical reinforcement and improvement of gas barrier properties have been explored in some AgNPs contained bio-nanocomposite films due to the physical attraction between the components and development of tortuous pathway of gas diffusion caused by the AgNPs [13].

Blending two or more polymers is one of the simplest methods to improve film properties by interacting with different polymers through physical entanglement to form the polymeric networks

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http://dx.doi.org/10.1016/j.ijbiomac.2015.07.007
0141-8130/

simultaneously or sequentially [4]. Generating physical hydrogel films through combinations of polyelectrolyte biomolecules with opposite charges offers distinct advantages arising from the strength of the multiple intermolecular associations. One of the most promising biopolymer pairs for the formation of a hydrogel is a protein-polysaccharide association. Mixing counter-charged biopolymers leads to formation of compound coacervation and polyionic (polyelectrolyte) complexes, and precipitates or gels to form supra-molecular structures under specific conditions of ionic strength, biopolymer ratio, and pH [14].

In the present study, we tested three biopolymers, agar, alginate, and collagen to prepare composite hydrogel films. Agar is a polysaccharide extracted from the *Gelidiaceae* and *Gracilariaceae* families of seaweeds and mainly composed of alternating D-galactose and 3, 6-anhydro-L-galactopyranose repeating units [15,16]. Agar is known to melt on heating and set on cooling and this cycle can be repeated for an indefinite number of times without compromising the mechanical properties of the gel. Such gel forming properties of agar make it a good candidate for blending with other biopolymers to enhance the mechanical properties of the blended hydrogels [4,17]. Alginate have attracted particular interest because of its extraordinary high water holding capacity and film forming ability under mild conditions at room temperature [18]. Alginate, an anionic polysaccharide of β-D-mannuronic acid and α-L-guluronic acid linked together with varying proportions of 1-4-linkages [19], is usually extracted from brown algae (Phaeophyceae) such as *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Ascophyllum nodosum*, and *Macrocystis pyrifera* [20]. Alginate hydrogels are mostly prepared by external gelation, using calcium ions as cross-linking agents. The cation interacts and binds with guluronate blocks of alginate chains, forming the gel network [18,21]. Collagen, a well-known fibrillar protein obtained from connective tissues of animals, has received considerable attention because of its abundance and many important biological functions such as tissue formation and cell adhesion with excellent biodegradability and biocompatibility [22]. Collagen has been widely used as biomaterials in a variety of applications such as wound dressing, scaffold materials for tissue engineering, drug delivery systems [23], orthopedics, and preparation of biopolymer composites.

The main objective of the present study was to prepare agar/alginate/collagen ternary blend hydrogel film by including antimicrobial additives such as silver nanoparticles (AgNPs) and grapefruit seed extract (GSE) to develop functional biohydrogel films for food packaging application.

2. Experimental

2.1. Materials

Food grade agar was purchased from Fine Agar Co., Ltd. (Damyang, Jeonnam, Korea). Na-alginate (Product No.: 37094-01) and collagen were procured from Kanto Chemical Co. (Tokyo, Japan) and MSC Co., Ltd. (Sungnam, Gyunggido, Korea), respectively. Glycerol was obtained from Daejung Chemicals & Metals Co., Ltd. (Siheung, Gyunggido, Korea). Grapefruit seed extract (GSE) was purchased from Food Additive Bank Co., Ltd. (Ansung, Gyunggido, Korea). Silver nitrate ($AgNO_3$) and sodium citrate were of analytical grade and used without further purification. Tryptic soy broth (TSB) and brain heart infusion broth (BHI), and agar powder were purchased from Duksan Pure Chemicals Co., Ltd. (Ansan, Gyunggido, Korea). Foodborne pathogenic microorganisms such as *Listeria monocytogenes* ATCC 15313 and *Escherichia coli* O157:H7 ATCC 43895 were obtained from Korean Collection for Type Culture (KCTC, Seoul, Korea). Both of these strains were grown in TSA and BHI agar medium and stored at 4 °C for further test. Fresh potatoes for packaging test were purchased from a local market.

2.2. Preparation of films

Agar/alginate/collagen (A/A/C) ternary blend films were prepared by using solvent casting method [4]. Film solution was prepared by dissolving 3 g of agar, alginate, and collagen powder (1 g of each) into 150 mL of distilled water with 0.9 g of glycerol as a plasticizer while mixing vigorously for about 25 min at 95 °C using a magnetic stirrer. The A/A/C and AgNPs composite film ($A/A/C^{AgNPs}$) solutions were prepared following the method described by Rhim et al. [12]. First, silver nitrate ($AgNO_3$) stock solution was prepared by dissolving 4.72 g of silver nitrate into 100 mL of distilled water with boiling for 1 h. For the reduction of the $AgNO_3$, 1 mL of the stock solution was mixed with 150 mL of distilled water and 2 mL of 1% sodium citrate solution, and boil the solution for about 1 to get greenish yellow silver nanoparticle solution. Then 3 g of agar, alginate, and collagen powder were dissolved into the AgNPs solution with 0.9 g of glycerol and followed the same procedure to prepare the film solutions. For the preparation of A/A/C with GSE ($A/A/C^{GSE}$) film solutions, 0.3 g of GSE (10 wt% of polymers) were added into 150 mL of distilled water and followed the same procedure to prepare the film solution.

All the film solutions were cast onto leveled glass plate (24 cm × 30 cm) coated with Teflon layer (Cole-Parmer Instrument Co., Chicago, IL, USA) and dried for about 24 h at room temperature and peeled off from the plate to get dried film. Film thickness was measured using a micrometer (Dial Thickness gauge 7301, Mitutoyo, Japan) with an accuracy of 0.01 mm. All film samples were preconditioned in a constant temperature humidity chamber set at 25 °C and 50% RH for at least 48 h before further test.

2.3. Surface color and transparency of films

Surface color of the film was measured using a Chroma meter (Konica Minolta, CR-400, Tokyo, Japan). A white standard color plate ($L = 97.75$, $a = -0.49$, and $b = 1.96$) was used as a background for color measurements. Hunter color (L, a, and b) values were averaged from five readings from each sample. The total color difference (ΔE) was calculated as follows:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5} \quad (1)$$

where ΔL, Δa, and Δb are difference between each color values of standard color plate and film specimen, respectively.

The optical properties of the films were tested by measuring UV/vis transmission spectrum of film samples. UV/vis transmission measurements were performed in the range of 200–700 nm using a UV/vis spectrophotometer (Model 8451A, Hewlett Packard Co., Santa Alara, CA, USA). The transparency of the films was evaluated as the percent transmittance at both UV (T_{280}) and visible (T_{660}) ranges measured at 280 and 660 nm, respectively.

2.4. Surface morphology and FT-IR analysis

Surface morphology of the films was observed using a field emission scanning electron microscopy (FE-SEM, S-4800, Hitachi Co., Ltd., Matsuda, Japan) operated at an acceleration voltage (V_{acc}) of 5.0 kV.

Fourier transform infrared (FT-IR) spectra of the film samples were obtained in the wavenumber of 4000–500 cm^{-1} using an attenuated total reflectance-Fourier transform infrared (ATR FT-IR) spectrophotometer (TENSOR 37 spectrophotometer with OPUS 6.0 software, Billerica, MA, USA).