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Preparation and characterization of agar/lignin/silver nanoparticles composite films with ultraviolet light barrier and antibacterial properties

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ABSTRACT

Lignin was used to prepare silver nanoparticles (AgNPs), and they were incorporated into agar-based films. The composite films were characterized using UV–visible spectroscopy, FE-SEM, FTIR, XRD, and TGA. The color, mechanical, water vapor barrier, and antibacterial properties of the composite films were also evaluated. The composite films exhibited characteristic light absorption peaks at 250–300 nm and 430 nm due to the lignin and AgNPs, respectively. The interaction of lignin and AgNPs with agar biopolymer was analyzed using FTIR. XRD results showed the characteristic peaks of crystalline AgNPs. Incorporation of lignin and AgNPs increased the mechanical, UV-light barrier, and water vapor barrier properties of the composite films. The loading of 1 wt% of AgNPs was the optimum concentration to improve the mechanical and water vapor barrier properties of the films. The films containing AgNPs exhibited antibacterial activity against food-borne pathogenic bacteria, *Escherichia coli* and *Listeria monocytogenes*.

1. Introduction

There have been serious concerns about the contamination of food by micro-organisms which can reduce the shelf-life of food as well as increase the risks of various food-borne infections causing serious illness to human beings (Devlieghere, Vermeiren, & Debevere, 2004). Traditionally, various physical and chemical preservation methods have been used in the food industry to reduce the spoilage of food, maintain food quality, and extend the shelf-life of food products. The most common method to control the growth of micro-organisms on the surface of the food is to apply antimicrobial agents or to dip the food materials into an antimicrobial solution (Sung et al., 2013). However, concerns on the diffusion of such antimicrobial agents to the inside of foodstuffs have limited the use of this technology. Also, recently increased consumer demands for the minimally processed and ready-to-eat fresh foods have inspired researchers to develop new technologies for securing food safety and maintaining food quality (Bhat, 2013). Packaging materials with antimicrobial function have been recognized as one of the most promising active packaging systems for extending shelf-life of food, maintaining food safety and quality, and improving storage stability by destroying or inhibiting the spoilage and pathogenic microorganisms (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011; Han, 2000). Antimicrobial packaging is often achieved by incorporation of antimicrobial agents into the packaging system. Some organic and inorganic materials have been used as antimicrobial agents for the antimicrobial packaging application (Kanmani & Rhim, 2014; Shankar, Teng, Li, & Rhim, 2015; Shankar, Teng, & Rhim, 2014). However, a low thermal stability of organic antimicrobial materials have limited their broad use in food packaging applications. In contrast, the high thermostability of inorganic antimicrobial agents such as metallic nanoparticles has opened the way for their use in the food packaging industries (Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012).

Among the metallic nanoparticles, silver nanoparticles (AgNPs) have been most widely used for the preparation of nanocomposite in the food packaging and biomedical applications due to their high

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surface area, unique optical, magnetic, electric, and catalytic properties with high thermal stability and broad-spectrum of antimicrobial activity (Duncan, 2011; Rhim, Park, & Ha, 2013). The antimicrobial effect of AgNPs is known to be greatly influenced by the size, shape, and method of preparation of the AgNPs (Lischer et al., 2011). The AgNPs synthesized by green methods are recognized as safe for the use in the food packaging applications. For the green synthesis of AgNPs, various biopolymers and biomolecules such as gelatin (Kanmani & Rhim, 2014), banana powder (Orsuwana, Shankar, Wang, Sothornvit, & Rhim, 2016), chitosan (Huang & Yang, 2004), vitamins (Shankar & Rhim, 2016), amino-acids (Shankar & Rhim, 2015), and plant extracts (Shankar, Chorachoo, Jaiswal, & Voravuthikunchai, 2014) have been used. As one of such biopolymeric materials with high potential for the preparation of AgNPs, lignin is interesting since it can be used not only as reducing and stabilizing agent for the AgNPs, but also as a filler for the preparation of nanocomposite films.

Lignin is a complex phenolic compound which is abundantly present in the cell wall of plants in association with cellulose and hemicellulose (Tuomela, Vikman, Hatakka, & Itavaara, 2000). Although lignin is chemically heterogeneous and structurally complex material, it possesses multiple functional groups such as reductive aliphatic hydroxyls, phenolic hydroxyls, and thiols, which can serve as reducing and stabilizing agents for the synthesis of silver nanoparticles. Thiols have shown high affinity to silver nanoparticles, hence facilitating its adsorption and capping onto particle surface (Laibinis et al., 1991). The polar sulfonate groups in lignin can also help to disperse the formed nanoparticles in aqueous solutions. Also, lignin possesses a strong UV light absorption property (Monties, 1991; Shankar, Reddy, & Rhim, 2015).

Recently, biopolymers from various natural resources have been used as eco-friendly packaging materials to substitute the non-biodegradable, petroleum-based plastic based packaging materials (Gimenez, Lopez de Lacey, Perez-Santín, Lopez-Caballero, & Montero, 2013). Among biopolymers, agar has been widely used for the preparation of biodegradable packaging films due to its good film forming property with abundance, renewability, and biocompatibility (Shankar & Rhim, 2016; Shankar, Reddy et al., 2015; Shankar, Teng et al., 2014). Agar is a hydrophilic polysaccharide extracted from the Gelidiaceae and Gracilariaceae families of seaweeds and mainly composed of alternating repeating units of D-galactose and 3, 6-anhydro-ß-galactopyranose (Tako, Higa, Medoruma, & Nakasone, 1999).

Therefore, the present study was aimed to develop agar-based biodegradable food packaging films incorporated with lignin and lignin capped silver nanoparticles. The films were characterized using various analytical techniques. The UV-light barrier, mechanical, thermal, water vapor barrier and antibacterial properties of the films were also evaluated.

2. Materials and methods

2.1. Materials

The food grade agar was obtained from Fine Agar Co., Ltd. (Damyang, Jeonnam, Korea). Glycerol and alkali-soluble low sulfonate lignin were procured from Sigma-Aldrich Co. (St. Louis, MO, USA). The lignin was dried at 80 °C for 6 h before use. Silver nitrate ($AgNO_3$), brain heart infusion broth (BHI), and tryptic soy broth (TSB) were obtained from Duksan Pure Chemicals Co., Ltd. (Ansan, Gyeonggi-do, Korea). *Escherichia coli* O157: H7 ATCC 43895 and *Listeria monocytogenes* ATCC 15313 were procured from the Korean Collection for Type Cultures (KCTC, Seoul, Korea).

2.2. Synthesis of agar/lignin/AgNPs composite films

Silver nanoparticles were synthesized using lignin as reducing and capping agents. 0.12 g of lignin (3 wt% based on agar) was dispersed in 150 mL of distilled water and stirred for 20 min at 90 °C using a magnetic stirrer. Different amounts of $AgNO_3$ (0.5, 1.0, 1.5, and 2 wt% based on agar) were added dropwise into the lignin solution and continued heating the mixture for 20 min. 1.2 g of glycerol (30 wt% based on agar) was added into the mixture as a plasticizer with vigorous stirring for 10 min followed by addition of 4 g of agar and heated continuously with stirring at 90 °C for 20 min using a hot plate. The fully solubilized film forming solution was cast evenly onto a leveled Teflon film-coated glass plate (24 cm × 30 cm) and allowed to dry at room temperature for about 48 h. The dried films were peeled off from the plate and conditioned in a humidity chamber set at 25 °C and 50% RH for 48 h before further analysis. Additionally, neat agar and agar with 3 wt% of lignin films were prepared by the same procedure except adding $AgNO_3$.

2.3. Characterization of agar/lignin/AgNPs composite films

2.3.1. Surface morphology and optical properties

The surface morphology of the neat agar, agar/lignin, and agar/lignin/AgNPs composite films was observed using a field emission scanning electron microscope (FE-SEM, S-4800, Hitachi Co., Ltd., Matsuda, Japan). The film samples were attached on the specimen holder, sputter coated with platinum, and the image was analyzed at an accelerating voltage of 3 kV.

The optical properties of the composite films were determined by measuring the absorption of light between 200 and 700 nm using UV–visible spectrophotometer (Mecasys Optizen POP Series UV/Vis, Seoul, Korea).

2.3.2. FTIR and XRD

Fourier transform infrared (FTIR) spectra of the composite films were measured using an attenuated total Reflectance-Fourier Transform infrared (ATR-FTIR) spectrophotometer (TENSOR 37 spectrophotometer with OPUS 6.0 software, Billerica, MA, USA). The FTIR spectra were recorded as 32 scans per samples at a resolution of 4 cm^{-1}.

X-ray diffraction (XRD) pattern of the films was analyzed using an X-ray diffractometer (PANalytical X' pert pro MRD diffractometer, Amsterdam, Netherlands). The film samples (2.5 cm × 2.5 cm) were placed on a glass slide, and the XRD spectra were recorded using Cu Kα radiation (wavelength of 0.1541 nm) and a nickel monochromator filtering wave at 40 kV and 30 mA. The diffraction pattern was recorded at $2\theta = 30–80°$ with a scanning speed of 0.4°/min at room temperature.

2.3.3. Thermogravimetric analysis

Thermal stability of the composite films was determined using a thermogravimetric analyzer (Hi-Res TGA 2950, TA Instrument, New Castle, DE, USA). The film samples were taken in a standard aluminum pan and heated from 30 to 600 °C at the rate of 10 °C/min under a nitrogen flow of 50 mL/min. A derivative form of TGA (DTG) was obtained and the maximum decomposition temperature (T_{max}) of the composite films was obtained from DTG curve.

2.3.4. Surface color and light transmittance

The Hunter color values (L, a, and b) of the composite films were measured using a Chroma meter (Minolta, CR-200, Tokyo, Japan). A white color standard plate ($L = 97.75$, $a = -0.49$, and $b = 1.96$) was used as a background for the color measurements. The total color difference (ΔE) was calculated as follows: