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Preparation of antimicrobial agar/banana powder blend films reinforced with silver nanoparticles

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ABSTRACT

Binary blend films of agar and banana powder (A/B) and A/B composite films reinforced with silver nanoparticles (A/B/AgNPs) were prepared using a solution casting method and their properties were characterized. The SEM micrographs and FT-IR results confirmed the formation of physical interactions between polymer matrices and nanofillers. Apparent surface color and transmittance of the composite film were greatly influenced not only by the mixing of banana powder with agar but also by the incorporation of AgNPs. The UV light absorption, water vapor barrier properties, and antioxidant activity of A/B blend films increased with the increase in the concentration of the banana powder, while the mechanical properties decreased. The A/B/AgNPs composite film exhibited distinctive antimicrobial activity against food-borne pathogenic bacteria, *Escherichia coli* and *Listeria monocytogenes* with stronger antibacterial activity against Gram-negative bacteria than Gram-positive bacteria. The binary blend of A/B films are expected to be used for the edible film or coating of foods and their nanocomposite films with antimicrobial activity have a potential to be used as food packaging material for maintaining the safety and extending the shelf life of packaged food.

1. Introduction

Recently, biopolymers from various natural resources such as starch, cellulose, agar, alginate, carrageenan, gelatin, soy protein, whey protein, and wheat gluten have been used as eco-friendly packaging materials for the substitute of non-biodegradable petroleum-based plastic based packaging materials (Shankar, Teng, Li, & Rhim, 2015a; Shankar, Reddy, & Rhim, 2015b; Gimenez, Lacey, Santin, Caballero, & Montero, 2013). As one of such 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 (Wang & Rhim, 2015). 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-β-galactopyranose (Gehrke, 1993; Tako, Higa, Medoruma, & Nakasone, 1999). High compatibility with other biopolymers of agar with good film-forming properties made it as a good candidate for blending with other biopolymers to enhance the properties of the blended films (El-Hefian, Nasef, & Yahaya, 2012; Varshney, 2007; Wang & Rhim, 2015). Various materials, such as protein (Wang & Rhim, 2015), nano-clay (Kanmani & Rhim, 2014a), nanocellulose (Shankar & Rhim, 2016), grapefruit seed extract (Kanmani & Rhim, 2014b), lignin (Shankar et al., 2015b), and metallic nanoparticles (Shankar & Rhim, 2015) have been blended with agar to improve the mechanical, water resistance, and functional properties of the films. However, to the best of our knowledge, the report on banana powder as a reinforcing agent in agar biopolymer is not available in the literature so far.

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Banana, *Musa sapientum* Linn. is a tropical fruit which contains a high amount of polysaccharide (starch 61–76% dry basis), proteins, and fat (Waliszewski, Aparicio, Bello, & Monroy, 2003). In addition, at the green stage, banana is a major source of macro-elements and it contains health-beneficial ingredients such as resistant starch and dietary fibers that have the potential to increase the hydrophobicity of polymers (Anyasi, Jideani, & Mchau, 2013; Pelissari, Andrade-Mahecha, Sobral, & Menegalli, 2013). Moreover, the polyphenolic compounds included in banana are expected to enhance the functional properties, to secure the food safety, and to extend the shelf-life of food (Pereira & Maraschin, 2015; Sothornvit & Pitak, 2007; Waliszewski et al., 2003). Banana peel extract has been used for the synthesis of silver nanoparticles for the test of their antimicrobial and free radical scavenging activities (Kokila, Ramesh, & Geetha, 2005).

Bionanocomposite packaging materials with antibacterial function is believed to be one of the promising active packaging materials to extend the shelf-life of food, maintain the food safety, quality, and to improve the storage period by destroying or inhibiting the food pathogenic microorganisms (Kanmani & Rhim, 2014a; Shankar, Teng, & Rhim, 2014a). Silver nanoparticles (AgNPs) have been most widely used for the preparation of nanocomposite in the food packaging and biomedical applications due to their high surface area, unique optical, magnetic, electric, catalytic, thermal stability, and broad-spectrum of antimicrobial properties. Instead of hazardous chemical reagents, various biopolymers such as gelatin (Kanmani & Rhim, 2014a), glucose, starch (Cheviron, Gouanvé, & Espuche, 2014; Vigneshwaran, Nachane, Balasubramanya, & Varadarajan, 2006), and chitosan (Huang & Yang, 2004), as well as plant extracts (Shankar Chorachoo, Jaiswal, & Voravuthikunchai, 2014b; Shankar, Jaiswal, Aparna, & Prasad, 2014c) have been used for the synthesis of AgNPs. Therefore, in the present study, banana powder was used as a reducing and stabilizing agent for the preparation of AgNPs.

The objectives of the present study were to prepare agar/banana powder binary blend films (A/B) and agar/banana powder blend films with AgNPs (A/B/AgNPs) and to characterize their properties for their potential use as food packaging application. Banana powder was aimed to blend with agar to improve the water barrier and functional properties such as ultraviolet (UV) screening effect, antioxidant, and antimicrobial activity of the A/B binary blend film. Banana powder is rich in carbohydrate composed of starch as the main constituent with good film-forming property (Waliszewski et al., 2003). A small amount of protein, ash, and fat presented in banana powder played an important role in the optical, physicochemical properties when used as a main or a part of the raw material to form a film (Pelissari et al., 2013). In addition, banana powder contained some phytochemicals such as tannins and terpenoids (β-carotene) as phytochemicals (Anyasi et al., 2013). Tannins are one of a polyphenolic compound found in unripe fruit that can provide antioxidant activity and help in reducing metal ions to nanoparticles (Pereira & Maraschin, 2015).

2. Materials and methods

2.1. Materials

Green banana (*M. sapientum* Linn.) at the age of 112–116 days after petal fall was obtained from the orchard of Kasetsart University, Khamphaengsaen Campus, Nakhonpathom, Thailand. Food grade agar was purchased from Yun Doo Co., Ltd. (Uijeongbu, Gyeonggi-do, Korea). Glycerol was procured from Daejung Chemicals & Metals Co., Ltd. (Siheung, Gyeonggi-do, Korea). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Tryptic soy broth (TSB) and brain heart infusion broth (BHI), agar powder, and silver nitrate ($AgNO_3$) were purchased from Duksan Pure Chemicals Co., Ltd. (Ansan, Gyeonggi-do, Korea). Foodborne pathogenic microorganisms, *Listeria monocytogenes* ATCC 15313 and *Escherichia coli* O157:H7 ATCC 43895, were obtained from Korean Collection for Type Culture (KCTC, Seoul, Korea). The test microorganisms were grown in BHI and TSB agar medium, respectively, and stored at 4 °C for further test.

2.2. Preparation of banana powder

Banana powder was prepared according to the method of Sothornvit and Pitak (2007). Green bananas were washed, peeled, sliced, and soaked into 0.01% of sodium chloride solution for 30 min. After drained the solution, the banana slices were dried at 70 °C for 12 h in a drying oven. Banana powder was obtained by grinding the dried banana slices using a hammer mill and passing through a 100-mesh sieve. The proximate composition of the banana powder was as follows:

moisture (8.17 ± 0.04%), starch (75.09 ± 0.43%), protein (10.79 ± 0.14%), fat (0.36 ± 0.07%), and ash (5.59 ± 0.31%).

2.3. Preparation of agar/banana blend films

The agar/banana binary blend films (A/B) with different blending ratio of agar and banana powder (4/0, 3/1, 2/2, 1/3, and 0/4) were using a solvent casting method (Kanmani & Rhim, 2014a). Film forming solution was prepared by dissolving 4 g of agar and banana powder with different ratio in 150 mL of distilled water, heating the suspension at 90 °C for 20 min with continuous stirring, and adding 1.2 g of glycerol (30 wt% of solid) as a plasticizer.

In addition, two more A/B blend composite films incorporated with AgNPs were prepared using two different blending film solution, i.e., A_2/B_2 and A_0/B_4. For the preparation of nanocomposite film forming solution, 1.5 mL of 100 mM aqueous solution of $AgNO_3$ was added dropwised into the A/B film solution to make a final concentration of 1 mM of silver and heated at 90 °C for 4 h with constant stirring.

All film forming solutions were cast onto the leveled Teflon film (Cole-Parmer Instrument Co., Chicago, IL, USA) coated glass plates (24 cm × 30 cm) and dried at room temperature for 48 h. The films were peeled off from the plate after drying and preconditioned in a humidity chamber (model FX 1077, Jeio Tech Co. Ltd., Ansan, Korea) controlled at 25 °C and 50% RH for at least 48 h. The films were designated as A_4/B_0, A_3/B_1, A_2/B_2, A_1/B_3, A_0/B_4, A_2/B_2/AgNPs, and A_0/B_4/AgNPs according to the mixing ratio of agar and banana powder as well as the incorporation of AgNPs.

2.4. Characterization of films

2.4.1. Morphology, surface color, and optical properties

Small pieces of film sample were placed on the SEM specimen holder and image analysis was performed using a field emission scanning electron microscopy (FE-SEM, S-4800, Hitachi Co., Ltd., Matsuda, Japan) with an accelerating voltage of 5.0 kV.

Surface color of the films was measured by Chroma meter (Konica Minolta, CR-400, Tokyo, Japan). A white standard color plate with Hunter color values, L = 97.75; a = −0.49; and b = 1.96 was used as a background for color measurements. Hunter color values of film samples were determined by calculating the average of five readings from each film. The total color difference (ΔE) was calculated as follows (Shankar & Rhim, 2016):