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Antimicrobial edible defatted soybean meal-based films incorporating the lactoperoxidase system

Hanna Lee, Sea C. Min*

Department of Food Science and Technology, Seoul Women's University, 621 Hwarangro, Nowon-gu, Seoul 139-774, Republic of Korea

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ABSTRACT

Antimicrobial films were developed using defatted soybean meal (DSM), a by-product left after crushing soybeans for oil, and the lactoperoxidase system (LPOS). Effects of the composition of the films on physical and antimicrobial properties of the films and the diffusion of antimicrobial hypothiocyanite ($OSCN^-$) from the LPOS in the films were investigated. The antimicrobial DSM film inhibited *Salmonella* (4 log CFU/cm^2) on agar media. Yellowness and elongation of the film increased, while lightness and the elastic modulus decreased as the concentration of protein increased in the film. The composition of the films did not affect their antimicrobial activity. The diffusion of antimicrobial $OSCN^-$ in the film increased with increasing concentrations of glycerol or protein in the film, storage temperature, or water activity of the food on which the film was applied, and thus the film can be formulated to achieve a necessary antimicrobial release rate. The composition of the DSM can be used to predict the physical and diffusion properties of the DSM-based antimicrobial films prepared from different DSMs. The DSM film coating did not significantly affect the sensory properties of sliced ham ($P > 0.05$), demonstrating the potential for the application to commercial ham products.

1. Introduction

Using antimicrobial edible films and coatings has been proposed as an innovative approach to decontaminate undesirable microorganisms, including *Salmonella*, during storage of ready-to-eat products (Eswaranandam, Hettiarachchy, & Johnson, 2004; Min, Harris, & Krochta, 2005a). Efforts have focused on the incorporation of natural antimicrobial agents in edible films and coatings (Alkan et al., 2011; Mayachiew, Devahastin, Mackey, & Niranjan, 2010; Sivarooban, Hettiarachchy, & Johnson, 2008). Lactoperoxidase system (LPOS) has been identified as a natural antimicrobial system in human secretions such as saliva, tear-fluid, and milk (Kussendrager & Van Hooijdonk, 2000) and suggested as a preservative for foods and pharmaceuticals (Bosch, Van Doorne, & De Vries, 2000). The antimicrobial activities of a whey protein-based film incorporating an LPOS against *Salmonella* have been reported (Min et al., 2005a; Min, Harris, & Krochta, 2005b).

Defatted soybean meal (DSM) is a by-product of soybeans crushed for oil. DSM is widely available with an annual production of about 150 million tons worldwide (Kikuchi & Furuta, 2009). Many research papers have reported edible films prepared using proteins extracted from soybeans (Cho & Rhee, 2002; Rhim, Gennadios, Handa, Curtis, & Milford, 2000; Tang, Jiang, Wen, & Yang, 2005). However, little information is available on the development of an edible film using DSM itself, i.e., not using the protein extracts from DSM. Although extraction and isolation of proteins is possible for film production, this would greatly increase film production costs.

This research is predicated on the need to develop a usable material from the agricultural by-product. To make use of DSM as a film-forming material, the effects of the composition of DSM on the properties of the film must be investigated because the biopolymer composition of DSM changes with species and location, as well as on an annual basis. In this research, model films were prepared from different ratios of the carbohydrates, proteins, and oil isolated from soybeans and DSM and the effects of composition on film properties were studied. Information obtained from this investigation may be useful in customizing DSM-based films (DSM films) with certain properties by manipulating the composition of the DSM materials and also in standardizing DSM films for commercial production.

The diffusion coefficients (*D*s) for antimicrobials in polymer matrices must be determined to understand diffusion phenomena in the films and thereby assess the ability of selected polymers to

* Corresponding author. Tel.: +82 2 970 5635; fax: +82 2 970 5977.
E-mail addresses: smin@swu.ac.kr, scmin2@gmail.com (S.C. Min).

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act as antimicrobial carriers (Franssen, Rumsey, & Krochta, 2004). Analytical solutions derived from Fick's second law of diffusion have adequately predicted *D* values for sodium chloride, potassium sorbate, acetic acid, and propionic acids in food products and edible films (Franssen et al., 2004; Ouattara, Simard, Piette, Begin, & Holley, 2000; Pflug, Fellers, & Gurevitz, 1967). Thus, the objectives of this research were to develop an antimicrobial film effective against *Salmonella* using DSM as a film base material and LPOS as an antimicrobial system; investigate the effects of film composition on color, tensile, moisture-barrier, and antimicrobial properties of the films; study the diffusion of $OSCN^-$ in the antimicrobial DSM films and model films with respect to the concentration of glycerol plasticizer in the DSM film, storage temperature, and the composition of the model films; and study the effect of the film coating on the glossiness, odor, hardness, cohesiveness, and flavor of sliced ham.

2. Materials and methods

2.1. Materials

The DSM was supplied from CJ Corp. (Seoul, Korea). Oil was extracted from soybean (*Glycine max*) by cold pressing and approximately 90% of the oil was removed from the bean using a mechanical seed crusher. The material produced during the pressing procedure consisted of irregularly shaped flakes approximately 1 mm thick and ranging in diameters from 0.2 to 2 cm. The meal remaining after oil extraction was not treated in any additional manner prior to use as the film base material. The DSM contained water, polysaccharides, proteins, lipids, and ashes of 10.0, 37.4, 45.7, 0.9, and 6.0 g/100 g meal, respectively. The composition analysis was conducted by Korea Food Research Institute (Sungnam, Korea). Soy protein and oil obtained from the same kind of soybean from which DSM was prepared were supplied from CJ Corp. Carbohydrate of DSM was obtained following the method of Jiang, Chen, and Xiong (2009). The soy protein, oil, and carbohydrate at different ratios were used to form films (model films). The LPOS was composed of lactoperoxidase (81 U/mg), glucose oxidase (20,000 U/mg), α-D-glucose, potassium thiocyanate, potassium iodide, and hydrogen peroxide. Lactoperoxidase, glucose oxidase, α-D-glucose. Hydrogen peroxide was purchased from Sigma–Aldrich (St. Louis, MO, USA); potassium thiocyanate and potassium iodide were purchased from Duksan Pure Chemical Co., Ltd (Ansan, Korea); glycerol used as a plasticizer was purchased from Fisher Scientific Inc. (Fair Lawn, NJ, USA). Polysorbate-20 (hydrophilic–lipophilic balance (HLB): 16.7), an emulsifier, was supplied by Ilshinwells Co., Ltd (Seoul, Korea). Sliced ham and sweet jelly of red beans were products of Lotte Ham (Seoul, Korea) and Haitai Confectionery & Foods (Seoul, Korea), respectively.

2.2. Lactoperoxidase system

The lactoperoxidase system was prepared following the method of Min and Krochta (2005). The weight ratio of lactoperoxidase, glucose, potassium thiocyanate, potassium iodide, and hydrogen peroxide was 1.00:0.35:108.70:1.09:1.74:2.17, which was selected based on those used by other researchers (Min & Krochta, 2005) and corresponded to those of raw milk (Denis & Ramet, 1989).

2.3. Film preparation

DSM was ground in a Scienceware Micro-Mill (Bel-Art Products, Pequannock, NJ, USA) and then sieved to provide a fine powder (<250 μm). DSM was exposed to ultraviolet light (40 W) for 1 h on a clean bench for microbial decontamination. A water suspension (10%) of DSM or a mixture of soybean carbohydrate, soy protein, and oil with the ratio (weight basis) of 88:22:1, 66:44:1, 44:66:1, or 22:88:1 was processed with a high-shear probe mixer (Ultra-Turrax, Model T25; IKA-Works, Inc., Wilmington, NC, USA) at 5000 rpm for 5 min. The homogenate was then treated by one or two passes of a high-pressure homogenizer at 172 MPa (D.O.S. Inc., Siheung, Korea). Glycerol was added in the homogenized hydrocolloid at 10, 20, 30, or 50% (w/w DSM). Polysorbate-20 was then mixed with the hydrocolloid (1%, w/w DSM), followed by homogenization at 5000 rpm for 5 min and heating at 90 °C for 5 min in a water bath. After cooling on ice, the colloid (film-forming solution) was degassed under vacuum. Films were cast by pipetting the degassed mixture onto Teflon plates (16.1 cm diameter) resting on a level granite surface. The amount of the film-forming solution pipetted was selected to produce a 0.1-mm-thick film. To prepare the antimicrobial film incorporating the LPOS, 1, 2, 3, 4, 5, and 10% (w/w) LPOS were added to the degassed film-forming solution and mixed for 5 min at 200 rpm in a shaker before casting. The film-forming solutions were dried for 3 days at 23 ± 2 °C/35 ± 5% relative humidity (RH). The dried films were peeled intact from the casting surface.

2.4. Color

A colorimeter (Chroma meter CR-400, Minolta Co. Ltd., Osaka, Japan) was used to measure *L*, *a*, and *b* values by CIELAB coordinates. The colorimeter was calibrated using a white tile (model CR-A43), Illuminate C, and a 10° standard observer. Total color difference (Δ*E*) was calculated using the determined *L*, *a*, and *b* values (Pranoto, Salokhe, & Rakshit, 2005).

2.5. Tensile and moisture-barrier properties

The American Society of Testing and Materials (ASTM) standard method D 882-01 (1997) was used to measure tensile strength (TS), percentage elongation at break (%*E*), and elastic modulus (EM) of films. Film samples were analyzed using a tensile property tester (Withlab. Co., Ltd, Anyang, Korea) operated with a 5-kg load cell, 50-mm grip distance, and a 30-mm/min crosshead speed. The Gravimetric Modified Cup Method based on ASTM E96-92 (McHugh, Avena-Bustillos, & Krochta, 1993) was used to determine water vapor permeability (WVP).

2.6. Bacterial strain

Microbial inhibition studies were conducted using *Salmonella* Typhimurium DT 104, obtained from the Food Science and Human Nutrition culture collection at Washington State University (Pullman, WA, USA). The strain was kept at −80 °C in CryoCare™ (KS70GR50, Key Science Products Inc., TX, USA) and thawed on ice before use. Tryptic soy agar (TSA) (BD Difco™, MD, USA) and tryptic soy broth (BD Difco™) were used as growth media.

2.7. Disk-covering test

A disk-covering test was conducted following the method of Lee, Noh, and Min (2012). The inoculum (100 μL) was spread on the surface of the TSA plate to produce a lawn of approximately 4 log CFU/cm^2. When the inhibition zone was exhibited or the growth was not visible below the film disk, the disk was aseptically placed in a sterile bag (29 mL, Nasco WHIRL-PAK®, Fort Atkinson, WI, USA.). The 0.1% peptone water was transferred in the bag and then the film in the bag was well destroyed by scraping using a disposable spreader. The content was transferred into a centrifuge tube, serially diluted, and plated (250 μL × 4) on TSA. The TSA plates were incubated for 48 h at 37 °C before colonies were counted. The