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Physical properties and antimicrobial activities of porcine meat and bone meal protein films containing coriander oil

Ji-Hyun Lee [a], Misun Won [b], Kyung Bin Song [a, *]

[a] Department of Food Science and Technology, Chungnam National University, Daejeon, 305-764, Republic of Korea
[b] Biomedical Genomics Research Center, KRIBB, Daejeon, 305-806, Republic of Korea

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ABSTRACT

Porcine meat and bone meal (MBM) protein films were prepared using proteins extracted from porcine meat and bone meal, and their mechanical properties, water vapor permeability, moisture content, and optical properties (transparency and opacity) were examined. In addition, coriander oil (CO) was incorporated into the MBM protein film to provide antimicrobial activity. Optimal conditions for the film preparation were 5 g of MBM protein, 2 g of fructose, 0.02 g of tannic acid, and 1 g of CO in 100 mL of film-forming solution. The inhibition zones of the MBM protein film containing CO against *Escherichia coli* O157:H7 and *Listeria monocytogenes* were 20.54 and 25.56 mm, respectively. These results suggest that the extracted proteins from porcine meat and bone meal can be used as a base material for edible film preparation and that the MBM films containing CO are useful for antimicrobial food packaging.

1. Introduction

As environmental pollution from the production of plastic packaging materials increases, more studies have been focusing on environment-friendly biodegradable packaging materials (Khwaldia, Arab-Tehrany, & Desobry, 2010). Particularly, proteins extracted from the by-products of the food industry have been used as packaging materials (Song, Shin, & Song, 2012; Song et al., 2013). In addition, the mechanical and barrier properties of protein-based films have been improved with the incorporation of various types of plasticizers or cross-linking agents (Limpan, Prodpran, Benjakul, & Prasarpran, 2010).

Porcine meat and bone meal (MBM) mixtures are obtained as a by-product of meat processing, and their nutritional profile is different depending on the method of production (Bolarinwa, Olukosi, & Adeola, 2012). Many countries, including France, Germany, and the USA, produce MBM, an inexpensive and protein-rich product containing high concentrations of Arg, Cys, Leu, Lys, Met, Phe, and Val (Cascarosa, Gea, & Arauzo, 2012; Parsons, Castanon, & Han, 1997). However, MBM is currently restricted as an animal feed by European Union legislation due to bovine spongiform encephalopathy, and most of MBM is discarded, causing environmental problems, such as pollution (Buckley, Penkman, Wess, Reaney, & Collins, 2012; Cascarosa et al., 2012).

Plasticizers make the films flexible and stretchable by decreasing the glass transition temperature of the films (Vieira, da Silva, dos Santos, & Beppu, 2011). In addition, cross-linking agents can form covalent bonds between protein molecules and improve the mechanical properties and water barrier properties of the films. Aldehydes, such as formaldehyde and glutaraldehyde, and less toxic agents, such as cinnamaldehyde, ferulic acid, and tannic acid, have been used as cross-linking agents (Balaguer, Gómez-Estaca, Gavara, & Hernandez-Munoz, 2011). Tannic acid, a phenolic cross-linker consisting of polygalloyl glucose residues, forms stable covalent bonds with protein polymers resulting in the improvement of the mechanical properties of the film (Kim, Silva, Kim, & Jung, 2010; Zhang et al., 2010).

Edible film can carry antimicrobials, which are directly applied to the surfaces of foods, and natural products, such as essential oils from plants (Gutierrez, Escudero, Batlle, & Nerín, 2009; Peng, Wu, & Li, 2013). Essential oils are generally recognized as safe and can be applied to films to improve their water barrier properties (Gutierrez, Barry-Ryan, & Bourke, 2009). Several essential oils such as cinnamon (*Cinnamomum verum*), coriander (*Coriandrum sativum*), clove (*Syzygium aromaticum*), and lemongrass (*Cymbopogon citratus*) have been used in protein-based films (Salgado, López-

* Corresponding author. Tel.: +82 42 821 6723; fax: +82 42 825 2664.
E-mail address: kbsong@cnu.ac.kr (K.B. Song).

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Caballero, Gómez-Guillén, Mauri, & Montero, 2013). It has been reported that the shelf-life of foods packaged with antimicrobial films containing essential oils or plant extract could be extended (Gutierrez, Barry-Ryan, et al., 2009; Gutiérrez, Escudero, Batlle, et al., 2009; Gutiérrez, Sánchez, Batlle, & Nerín, 2009; Gutiérrez, Batlle, Andújar, Sánchez, & Nerín, 2011; Nerín, 2012; Song et al., 2012). Particularly, coriander oil (CO), mainly composed of linalool, geraniol, limonene and camphor, has antimicrobial activity (Nurzyńska-Wierdak, 2013). Therefore, the objectives of this study were to extract proteins from MBM, prepare an antimicrobial MBM film containing CO, and evaluate the antimicrobial activity of the MBM film.

2. Materials and methods

2.1. Materials

Porcine MBM was purchased from a local market (Daejeon, Korea). For the preparation of MBM, unmarketable portions such as bones and offal from slaughtered pigs were heated at 130 °C for 90 min, passed through an expeller to eliminate the fat, and dried. The obtained porcine MBM (44.8% protein, 37.9% ash, 13.1% fat, 2.9% moisture, and 1.3% fiber) was ground and passed through a sieve (0.595 mm). Sodium chloride and ammonium sulfate were purchased from Daejung Chemicals & Metals Co. (Siheung, Korea). Plasticizers (glycerol, fructose, sorbitol, and sucrose) and cross-linking agents (cinnamaldehyde, tannic acid, and ferulic acid) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). CO extracted from the seeds of coriander, which mainly consists of monoterpene hydrocarbon, oleic acid, and phytol, was obtained from The Certification Academy for Holistic Aromatherapy (Seoul, Korea).

2.2. Extraction of porcine MBM protein

Extraction of proteins from MBM was performed according to the method described by Garcia and Phillips (2009) with a slight modification. For the preparation of MBM protein, MBM was treated with five volumes of 0.1 mol/L NaCl solution for 30 min. The mixture was then homogenized using a homogenizer (IKA, Ultra-Turrax T25, Staufan, Germany) at 10,000 rpm for 30 min and a sonicator (Model-GE 750, Sonics & Materials, Newtown, CT, USA) for 15 min. The soluble fraction was obtained by heating the sample at 60 °C for 3 h, followed by centrifugation and precipitation with 70% ammonium sulfate. The solution was dialyzed using regenerated cellulose dialysis tubing (MWCO, 3.5 kDa, Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) and lyophilized.

2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed with a gel (12.5%) containing a final concentration of 8 mol/L urea (Jeon, Ruzicka, Cho, Li, & Kim, 2011). The protein sample solution was mixed with sample buffer (1 mol/L Tris–HCl, 50 mL glycerol/100 mL solution, 10 g SDS/100 g solution, 1 g bromophenol blue/100 g solution, and 8 mol/L urea) at a 1:1 ratio (v/v). The mixture was heated at 95 °C for 10 min, and the samples were loaded onto the gel. After running the gel at a constant current of 20 mA, it was stained with Coomassie blue R-250 and destained.

2.4. Film preparation

MBM (5 g) was dissolved in 100 mL of distilled water. To dissolve the solution completely, it was stirred at room temperature for 30 min and homogenized using a homogenizer at 10,000 rpm for 5 min. The mixture was degassed under a vacuum for 5 min and then heated at 70 °C for 30 min. After heating, plasticizers, cross-linking agents, essential oils, and emulsifiers (Tween #20) were added and stirred for 10 min. The filtrate (80 mL) was poured onto a Teflon coated plate and then dried at room temperature for 18 h. The dried films were peeled off and conditioned in an environmental chamber for 48 h.

2.5. Mechanical properties

Film thickness was measured using a micrometer (Mitutoyo, Model No. 2046-08, Tokyo, Japan). Tensile strength (TS) and elongation at break (E) were evaluated with an Instron (M250–2.5 CT, The Testometric Company Ltd., Lancashire, UK). Samples were cut into uniform size (2.54 × 10 cm) and stored at 25 °C for 48 h before the test. Initial grip distance was 5 cm and cross-head speed was 50 cm/min. Five replicates for each film were used for the test.

2.6. Water vapor permeability (WVP)

Water vapor permeability (WVP) was measured using the ASTM Method E96-95 (1995) with a modification. A polymethylacrylate cup (20 mL) with 18 mL of distilled water was covered with MBM film (2 × 2 cm) and stored in an environmental chamber at 25 °C and 50% RH. The weight loss of the cup was measured every hour up to 8 h, and the WVP was determined in triplicate.

2.7. Transparency and opacity

Opacity was evaluated with a colorimeter (Minolta, CR-400, Tokyo, Japan) according to the method of Fakhoury et al. (2012). After calibration, three samples of each film were measured with white and black backgrounds. The opacity value was calculated by following equation:

$$Op = Opb/Opw \times 100,$$

where Opb = opacity of the MBM film against a black background and Opw = opacity of the MBM film against a white background.

To measure transparency, the films were cut to 2.5 × 1 cm, and the absorbance was measured at 600 nm using a spectrophotometer (UV-2450, Shimadzu Corporation, Kyoto, Japan). The transparency value was calculated according to the equation described by Han and Floros (1997).

2.8. Moisture content (MC)

MC of the film was measured according to the ASTM D644-99 method (1999). The samples (2 × 2 cm) were dried in an oven at 110 °C for 24 h after recording the initial weight. After drying, the dry weight of the films was determined, and MC was calculated.

2.9. Differential scanning calorimeter (DSC)

DSC was performed using a Mettler Toledo DSC 1 (Mettler Toledo, Columbus, OH, USA). The film sample (4.5 ± 0.1 mg) was placed and sealed into a sample pan and heated at a rate of 10 °C/min from −60 to 300 °C. An empty aluminum pan was used as a reference.

2.10. Agar diffusion test for antimicrobial activity

To measure the antimicrobial activity of MBM films containing CO, a disc diffusion test was performed using the method described