Journal of Food Engineering 120 (2014) 183–190

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Development of antimicrobial defatted soybean meal-based edible films incorporating the lactoperoxidase system by heat pressing

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ARTICLE INFO

Article history:
Received 15 May 2013
Received in revised form 23 July 2013
Accepted 24 July 2013
Available online 6 August 2013

Keywords:
Antimicrobial film
Heat pressing
Soybean meal
Lactoperoxidase system
Salmonella

ABSTRACT

Antimicrobial edible films were developed using defatted soybean meal (DSM), a lactoperoxidase system (LPOS), and heat pressing. DSM-based films were formed at 70–90 °C and 40–50 MPa for 3 min, varying with the concentrations of DSM, xanthan, glycerol, and water in the formulation. The tensile strength, elongation, elastic modulus, and moisture vapor permeability of the DSM films were 1.2–5.4 MPa, 0.7–19.5%, 19.6–505.4 MPa, and 2.6–6.8 g mm kPa^{-1} h^{-1} m^{-2}, respectively. The LPOS-DSM film inhibited *Salmonella* Typhimurium by 1.5 log CFU/disk. The diffusion coefficients for the diffusion of antimicrobial hypothiocyanite ($OSCN^-$) in the LPOS-DSM film coated on ham and a gel foodwere $1.8–10.7 \times 10^{-15}$ m^2 - s^{-1}. As the water activity of the food or storage temperature increased, the coefficient also increased. These results demonstrated the feasibility of producing edible films from DSM on a large scale, which can potentially be used as a food coating to decontaminate *S.* Typhimurium on food surfaces by controlled release of antimicrobials.

1. Introduction

Post-processing protection using antimicrobial edible films and coatings has been proposed as an innovative approach that can be applied to ready-to-eat (RTE) products to minimize or prevent the growth of pathogenic microorganisms, including *Salmonella*, during storage (Eswaranandam et al., 2004; Min et al., 2005). Direct surface application of antibacterial substances onto food by dipping, dusting, or spraying has limited benefits, because the substances can immediately react with food components or rapidly diffuse into the food. Application of edible films containing antimicrobial substances has advantages over the direct application of antibacterial substances onto food, because edible films can be designed to slow the diffusion of the substances to the surfaces of the film coating and coated food and thus maintain necessary concentrations of the substances at both surfaces for a longer period than when the direct application of the substances is applied (Min et al., 2008). Biologically derived antimicrobial materials have gained interest in the food industry, because of their greater acceptance in the growing natural foods market (Hoover and Steenson, 1995). Thus, efforts have focused on incorporating such materials in edible films and coatings (Alkan et al., 2011; Bahram et al., 2013).

Lactoperoxidase system (LPOS) is a natural antimicrobial system in human secretions, such as saliva, tear fluid, and milk (Kussendrager and van Hooijdonk, 2000). The use of LPOS has been suggested as a preservative in foods and pharmaceuticals (Bosch et al., 2000). Antimicrobials in LPOS, such as hypothiocyanite ($OSCN^-$) and hypothiocyanous acid (HOSCN), oxidize the sulphydryl (SH) groups of microbial enzymes and other proteins, resulting in the inhibition of microorganisms (Kussendrager and van Hooijdonk, 2000). The antimicrobial activity of LPOS-incorporating whey protein-based films against *Salmonella* has been reported (Min et al., 2005).

Biopolymer materials have potentially many applications as replacements for synthetic plastics, considering their renewable and biodegradable characteristics (Cutter, 2006). Production of edible films from some agricultural process byproducts, including apple peel (Sablani et al., 2009), potato peel (Kang and Min, 2010), and defatted mustard seed meal (Kathleen et al., 2012), has been reported previously. These papers report the use of whole byproducts to form edible films, not the use of components extracted or isolated from the byproducts. The use of whole material would reduce the cost of manufacturing biopolymer films.

Diffusion coefficients (D) for antimicrobials in films must be determined to understand diffusion phenomena in polymer matrices and to assess the ability of selected polymers to act as antimicrobial carriers (Franssen et al., 2004). Analytical solutions derived from Fick's second law of diffusion have adequately predicted D coefficients for thiocyanate (SCN^-) and hypothiocyanite ($OSCN^-$) ions in whey protein-based films containing LPOS (Min et al., 2007).

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http://dx.doi.org/10.1016/j.jfoodeng.2013.07.035

Casting based on natural drying is a common method of preparing biopolymer films for research. However, the casting does not lend itself to large-scale production. Little research has been published on the production of edible films using unit operations that can be extended to commercial production. Moreover, no report of production of edible films directly from agricultural byproducts using such processes has been published. Thus, the objectives of this study were to (1) develop edible DSM-based films (DSM films) and antimicrobial DSM films incorporating LPOS (LPOS-DSM films) using heat pressing; (2) evaluate the antimicrobial activity of LPOS-DSM films against *Salmonella* Typhimurium; and (3) assess the diffusion of the antimicrobial $OSCN^-$ of the LPOS within LPOS-DSM films.

2. Materials and methods

2.1. Materials

The DSM was supplied by CJ Corp. (Seoul, Korea). Oil was extracted from soybeans (*Glycine max*) by cold pressing; 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 diameter from 2 to 20 mm. The meal remaining after oil extraction was not treated further 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. This compositional analysis was conducted by the Korea Food Research Institute (Sungnam, Korea).

The LPOS consisted of lactoperoxidase (LPO) (81 U/mg), glucose oxidase (GO) (20,000 U/mg), α-D-glucose, potassium thiocyanate (KSCN), potassium iodide (KI), hydrogen peroxide (H_2O_2), and phosphate buffer (pH 7.2). LPO, GO, α-D-glucose, H_2O_2, and phosphate buffer were purchased from Sigma–Aldrich (St. Louis, MO); KSCN and KI were purchased from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). Glycerol, used as a plasticizer, was purchased from Fisher Scientific Inc. (Fair Lawn, NJ). Xanthan, a crosslinker, was purchased from Wha Cheon Co., Ltd. (Seongnam, Korea). Polysorbate 20 (hydrophilic-lipophilic balance (HLB): 16.7), an emulsifier, was supplied by Ilshinwells Co., Ltd. (Seoul, Korea). The sliced ham and sweet jelly of red beans were products of Lotte Ham (Seoul, Korea) and Haitai Confectionary & Foods (Seoul, Korea), respectively.

2.2. Lactoperoxidase system

The LPOS solution was prepared following the method of Min and Krochta (2005). A modification was made to the concentrations of LPO in the system; the concentration of LPO was reduced from 0.7% (w/w LPOS) to 0.1% (w/w LPOS). The modified LPOS demonstrated an antimicrobial effect against *S.* Typhimurium not significantly different from that reported by Min and Krochta (2005). The weight ratios of the LPOS components were 1.0:500.0:1.6:5.0:8.0:10.0:500.0, in the order LPO, α-D-glucose, GO, KSCN, KI, and H_2O_2, and phosphate buffer.

2.3. Film preparation

DSM was ground in a Scienceware Micro-Mill (Bel-Art Products, Pequannock, NJ) and then sieved to provide a fine powder (<250 μm). The DSM powder mixtures (26–59% (w/w, wet basis (wb)), xanthan (10–90% (w/w DSM)), glycerol (0–16% (w/w, wb)), and water (29–41% (w/w, wb)) were prepared using a mortar and pestle. The mixture was heat-pressed (MH-15, Masada Seisakusho Co., Ltd., Tokyo, Japan) between two aluminum plates (250 mm × 250 mm) for 3 min at 70, 80, and 90 °C and at 40 and 50 MPa. Xanthan, glycerol, and water were used at 10–90% (w/w DSM), 0-16% (w/w, wb), and 29–41% (w/w, wb), respectively, to investigate the effects of film composition on its physical properties (Table 1). Adding xanthan at <10% formed cracked and discontinuous films. The additions of the glycerol plasticizer >16% and water at >41% resulted in sticky and wet films at 10 to 90% xanthan. Continuous films were not formed with <29% water at 10-90% and 0–16% xanthan and glycerol, respectively. The concentration of DSM in the film formulation was determined to make the total concentration 100% (w/w, wb). The heat-press temperature and pressure conditions were determined with ranges that allowed the formation of smooth and continuous films. LPOS-DSM films were prepared by mixing the film formulation with the LPOS at 10, 15, 20, and 25% (w/w, dry basis (db)). Adding >25% LPOS did not form a continuous film. The LPOS-DSM film-forming material was heat pressed at 90 °C and 40 MPa for 3 min. The film was peeled off the aluminum plate and stored in a chamber equilibrated at 50 ± 2% relative humidity (RH) before testing.

2.4. Color

A colorimeter (CR-400, Konica Minolta Sensing Inc., Osaka, Japan) was used to measure L, a, and b values by CIELab coordinates. The colorimeter was calibrated using a white tile, Illuminate C, and a 10° standard observer.

2.5. Tensile properties

The American Society of Testing and Materials standard method D882-01 (ASTM, 2002) was used to measure tensile strength (TS), percentage elongation at break (%E), and the elastic modulus (EM) of the 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.

2.6. Water vapor permeability

The Gravimetric Modified Cup Method, based on ASTM E96-92 (McHugh et al., 1993), was used to determine water vapor permeability (WVP). The fan speed in the cabinets was set to achieve an air velocity of 152 m/min to ensure uniform RH throughout the cabinets.

Table 1
Experimental variables used to determine the effects of the composition of film-forming material on the physical properties of the films.

Exp. No.	Actual level			Coded level		
	Xanthan % (w/w DSM) (% (w/w, wet basis wb))	Glycerol (% (w/w, wb))	Water (% (w/w, wb))	Xanthan (% (w/w DSM))	Glycerol (% (w/w, wb))	Water (% (w/w, wb))
1	10 (6)	8	29	−1	0	−1
2	90 (30)	8	29	1	0	−1
3	50 (18)	16	29	0	1	−1
4	50 (15)	16	41	0	1	1
5	10 (6)	0	35	−1	−1	0
6	10 (4)	16	35	−1	1	0
7	50 (19)	8	35	0	0	0
8	90 (24)	8	41	1	0	1
9	50 (20)	0	41	0	−1	1
10	90 (23)	16	35	1	1	0
11	50 (24)	0	29	0	−1	−1
12	90 (31)	0	35	1	−1	0
13	10 (5)	8	41	−1	0	1