Journal of Food Engineering 158 (2015) 30–38

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Quantitative risk assessments of the effect of an edible defatted soybean meal-based antimicrobial film on the survival of *Salmonella* on ham

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ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 4 December 2014
Accepted 1 March 2015
Available online 7 March 2015

Keywords:
Edible antimicrobial film
Quantitative risk assessment
Monte Carlo simulation
Salmonella
Ham

ABSTRACT

An edible antimicrobial film was prepared with defatted soybean meal (DSM) and the lactoperoxidase system (LPOS) (LPOS–DSM films) by heat pressing a film-forming mixture at 90 °C and 40 MPa for 3 min. The film was applied to ham slices and the concentration of hypothiocyanite ($OSCN^-$) was monitored over time. Based on the data obtained, a model was developed that predicted that a film containing $OSCN^-$ at 0.66 mg/g could provide 42.9 h of protection against 2.8 log CFU/g of *Salmonella* Typhimurium. A Monte Carlo simulation-based analysis estimated that coating ham with the LPOS–DSM film would decrease the probability of occurrence of >1 case of salmonellosis per year by 90.7%, associated with consumption of ham. The methods of quantitative risk assessments developed in this study may also be useful for evaluating other edible antimicrobial films.

1. Introduction

Outbreaks of foodborne disease associated with the consumption of ready-to-eat (RTE) foods have increased with increasing RTE food consumption (Ganan et al., 2013; SanŕAna et al., 2014). *Salmonella* has been identified as one of the pathogens causing foodborne disease from the consumption of RTE meat products. The incidence of salmonellosis related to RTE meat products was reported to be 24.6% (CDC, 2014).

Foodborne disease outbreaks from consumption of RTE ham products are primarily induced by cross- and post-contamination with pathogens from process equipment and personnel during processing and consumers after the product package is opened (Lianou et al., 2007). The surface of RTE meat products is the primary point of contamination during cutting and slicing processes in the plant and at home (Cabeza et al., 2007).

The potential of applying antimicrobial film-coatings to prevent post-processing-surface contamination of foodborne pathogens, including *Salmonella* Typhimurium, *Listeria innocua*, *L.* monocytogenes on ham products has been demonstrated (Lee and Min, 2013a; Santiago-Silva et al., 2009; Ye et al., 2008). A cellulose-based antimicrobial film incorporating pediocin reduced *L. innocua* on sliced ham by 2 log units after 15 days of storage at 12 °C (Santiago-Silva et al., 2009). A chitosan film impregnated with *Zataria multiflora* Boiss essential oil (5 and 10 g/kg) and grape seed extract (10 g/kg) resulted in a 0.5–1.0 log reduction in *L. monocytogenes* in RTE mortadella-type sausages after 6 days of storage at 4 °C (Moradi et al., 2011). Lauric arginate-coated polylactic acid films inhibited *L. monocytogenes* and *S.* Typhimurium on ham by 2–3 log units after 7 days of storage at 4 °C (Theinsathid et al., 2012). A natural antimicrobial lactoperoxidase system (LPOS)-incorporating defatted soybean meal (DSM)-based edible film (LPOS–DSM film) was used to decontaminate *Salmonella* on sliced ham; the sensory properties of the ham were unaffected by the film (Lee and Min, 2013a). The antimicrobial hypothiocyanite ions ($OSCN^-$) in LPOS have been used as a chemical marker for the quantification of antimicrobials in LPOS (Lee and Min, 2013a, 2013b; Min et al., 2007). We hypothesize that the release profile of $OSCN^-$ from the antimicrobial LPOS–DSM film and the requirements of the films can be predicted using a mathematical model of $OSCN^-$ diffusion with an experimentally determined inhibitory antimicrobial concentration of $OSCN^-$ and diffusion parameters. In this study, the requirements include the persistence of the antimicrobial agent ($OSCN^-$) at or above the inhibitory concentration against *S.* Typhimurium and the necessary values for the initial concentration of the antimicrobial agent and the film-coating thickness required for inhibition of *S.* Typhimurium over a selected period of time.

Most current food chain models are based on the quantitative microbial risk assessment (QMRA) methodology (Smid et al., 2010). Predictive microbiology is important for performance of QMRA (Poschet et al., 2004). QMRA principles with predictive microbiology and scenario analysis can provide an objective

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http://dx.doi.org/10.1016/j.jfoodeng.2015.03.002
0260-8774/

evaluation of the safety features of the production process, including antimicrobial concentration and decontamination time, and allow the manufacturer to predict outcomes before implementation (Puerta-Gomez et al., 2013; Smid et al., 2010). QMRA models are frequently studied by means of a Monte Carlo simulation using input values derived from their probability distributions and generating outputs by propagating random values through the system (Rigaux et al., 2013). The Monte Carlo simulation involves random sampling from each probability distribution in the model to produce a large number of scenarios, resulting in a risk distribution (Puerta-Gomez et al., 2013).

A tremendous effort has been made over the last decade to develop edible antimicrobial films and coatings to improve food safety and shelf life. Nonetheless, no research paper reports the prediction of the effectiveness of edible antimicrobial films and coatings on food products, including RTE meat products. QMRA may be crucial for application of antimicrobial films to food products and to ensure delivery of safe products to the consumer. Thus, the objective of this study was to develop the methods of the QMRA, which can be useful for the application of the antimicrobial LPOS–DSM film to RTE meat products. The risk assessments were conducted in this study to (1) predict the persistence of the antimicrobial precursor ($OSCN^-$) at or above the inhibitory concentration for *S.* Typhimurium in the LPOS–DSM film, as well as the initial concentration of $OSCN^-$ in the film and the film thickness required for inhibition over a given period of time, using the antimicrobial $OSCN^-$ diffusion parameters determined for the diffusion of antimicrobial $OSCN^-$ in the LPOS–DSM film; and (2) predict the effect of the antimicrobial film application on the probability of salmonellosis from consumption of sliced ham and the major variables associated with the risk of salmonellosis, using a Monte Carlo simulation inputting the data mainly obtained from literature.

2. Materials and methods

2.1. Predicting the requirements of the antimicrobial LPOS–DSM film

2.1.1. Ham samples

Both sliced and non-sliced ham products (enNature, Lotte Foods, Seoul, Korea) were purchased at a local supermarket. They were commercially sterilized products and were stored at 4 °C before use. Both ham products were labeled 'no preservatives' and contained 96% pork and ~4% water, salt, sugar, celery powder, acidulant, and vitamin C in descending order. The slice thickness was 0.8 mm. The ham slice sample was cut to 4-cm diameter using a cheese borer and knife sterilized with 70% (v/v) ethanol. Unsliced ham, used to simultaneously determine the diffusion and partition coefficients, was cut into circular cylinders with a diameter of 4.0 cm and a height of 2.5 cm using the cheese borer and knife.

2.1.2. Film preparation

The LPOS–DSM films were prepared following the method of Lee and Min (2013b). 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 DSM produced during pressing consisted of irregularly shaped flakes approximately 1 mm thick and ranging from 0.2 to 1.5 cm in diameters. DSM was ground in a food processor (MCH-702, Tongyang Magic Inc., Seoul, Korea) and sieved to yield a fine powder (<250 μm). 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). The LPOS was prepared following the method of Min et al. (2005). The lactoperxidase (81 U/mg) from bovine milk was purchased from Sigma–Aldrich (St. Louis, MO, USA). Water (31.9%, w/w) was blended with the mixture of DSM powder (26.6%), xanthan (13.2%), glycerol (6.0%), and LPOS solution (22.3%) 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 90 °C and 40 MPa to form a LPOS–DSM film.

2.1.3. Determination of diffusion and partition coefficients (D_1, D_2, and K_{12})

Previously prepared DSM films were trimmed into 4.0-cm-diameter circular disks and placed on the top surfaces of the cylindrical ham samples (4.0-cm diameter, 2.5-cm height). All sides except for the top surface were coated with paraffin wax. The paraffin wax was applied in a melted state using a paintbrush to ensure one-dimensional diffusion and prevent moisture loss. The film-coated ham samples were stored at 4 °C for 10, 25, 48, 120, and 168 h. A temperature of 4 °C was selected because it represents good practice for storage of ham products, including unpackaged products. The wax coating and the film disk were removed from each ham sample after storage. The peeled samples were wrapped in Saran Wrap and aluminum foil and placed in a freezer (−80 °C) for 3 h. After hardening, six 3-mm slices were cut from frozen samples starting at and parallel to the surface that had been coated with the film disk using a cryosectioning device (Leica CM3050 S, Leica Microsystems, Wetzlar, Germany). The $OSCN^-$ concentrations of the removed film disk and the ham slices were determined as described in Section 2.1.5.

The following model from Fick's second law for one-dimensional diffusion in coated food (Min et al., 2008) was applied to express diffusion in the coated ham sample.

$$\overline{C}_{2ab}(t) = \frac{C_0 K_{12}(1+\gamma)}{2(b-a)} \sum_{n=0}^{\infty} \{\gamma^n [I_1 - I_2]\} \tag{1}$$

where

$$I_1 = \frac{2\sqrt{D_1 t}}{\mu}\left[-\frac{2nl+\mu(a-l)}{2\sqrt{D_1 t}} erfc\left(\frac{2nl+\mu(a-l)}{2\sqrt{D_1 t}}\right) + \frac{1}{\sqrt{\pi}} \exp\left(-\left(\frac{2nl+\mu(a-l)}{2\sqrt{D_1 t}}\right)^2\right) + \frac{2nl+\mu(b-l)}{2\sqrt{D_1 t}} erfc\left(\frac{2nl+\mu(b-l)}{2\sqrt{D_1 t}}\right) - \frac{1}{\sqrt{\pi}} \exp\left(-\left(\frac{2nl+\mu(b-l)}{2\sqrt{D_1 t}}\right)^2\right)\right] \tag{2}$$

$$I_2 = \frac{2\sqrt{D_1 t}}{\mu}\left[-\frac{(2n+2)l+\mu(a-l)}{2\sqrt{D_1 t}} erfc\left(\frac{(2n+2)l+\mu(a-l)}{2\sqrt{D_1 t}}\right) + \frac{1}{\sqrt{\pi}} \exp\left(-\left(\frac{(2n+2)l+\mu(a-l)}{2\sqrt{D_1 t}}\right)^2\right) + \frac{(2n+2)l+\mu(b-l)}{2\sqrt{D_1 t}} \times erfc\left(\frac{(2n+2)l+\mu(b-l)}{2\sqrt{D_1 t}}\right) - \frac{1}{\sqrt{\pi}} \exp\left(-\left(\frac{(2n+2)l+\mu(b-l)}{2\sqrt{D_1 t}}\right)^2\right)\right] \tag{3}$$

where C_0 = the initial concentration of $OSCN^-$ in the film; $a = x$ coordinate of the front edge of the slice; $b = x$ coordinate of the rear edge of the slice; t = time; l = thickness of the film;

$$\mu \equiv \sqrt{\frac{D_1}{D_2}}; \quad \gamma \equiv \frac{\mu - K_{12}}{\mu + K_{12}}$$

D_1 = $OSCN^-$ diffusion coefficient in the LPOS–DSM film; D_2 = $OSCN^-$ diffusion coefficient in ham; and K_{12} = partition coefficient value for $OSCN^-$ at the film/ham interface.