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Anti-listeria activity of poly(lactic acid)/sawdust particle biocomposite film impregnated with pediocin PA-1/AcH and its use in raw sliced pork

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ABSTRACT

A novel poly(lactic acid) (PLA)/sawdust particle (SP) biocomposite film with anti-listeria activity was developed by incorporation of pediocin PA-1/AcH (Ped) using diffusion coating method. Sawdust particle played an important role in embedding pediocin into the hydrophobic PLA film. The anti-listeria activity of the PLA/SP biocomposite film incorporated with Ped (PLA/SP + Ped) was detected, while no activity against the tested pathogen was observed for the control PLA films (without SP and/or Ped). Dry-heat treatment of film before coating with Ped resulted in the highest Ped adsorption (11.63 ± 3.07 μg protein/cm^2) and the highest anti-listeria activity. A model study of PLA/SP + Ped as a food-contact antimicrobial packaging on raw sliced pork suggests a potential inhibition of *Listeria monocytogenes* (99% of total listerial population) on raw sliced pork during the chilled storage. This study supports the feasibility of using PLA/SP + Ped film to reduce the initial load of *L. monocytogenes* on the surface of raw pork.

1. Introduction

Listeria monocytogenes is a foodborne pathogen that causes a severe disease called listeriosis (Alves et al., 2006; Gialamas et al., 2010). *L. monocytogenes* has been listed in the top five highest ranking pathogens with respect to the total case of food-borne illnesses in the United States (Theinsathid et al., 2012) and is a major concern in food industries (Farber and Peterkin, 1991). This pathogenic bacterium is of major concern in a wide variety of foods, especially in chilled meat and ready-to-eat (RTE) meat products due to its ability to survive and grow at refrigeration temperatures (Schlech, 2000; Ye et al., 2008a, 2008b). In order to reduce healthy risk for the consumer from *L. monocytogenes*, criteria or recommendations for tolerable levels of *L. monocytogenes* in processed foods have been established. The USA and Thailand practice "zero tolerance", while Canada and France apply different norms according to the foodstuff (Thévenot et al., 2006).

To guarantee food safety through the inhibition of *L. monocytogenes*, the use of bacteriocins and other biologically derived antimicrobials with anti-listeria activity in packaging material have received a considerable attention (Coma, 2008; Lara-Lledó et al., 2012; Min et al., 2010; Sánchez-González et al., 2013). Thus, during the last decades, innovative bioactive films enriched with bacteriocins have been developed. The most-commonly studied antimicrobial agents for applications in this sense are nisin (Cao-Hoang et al., 2010; Guiga et al., 2010; Hoffman et al., 2001; Janes et al., 2002; Jin et al., 2009; Ko et al., 2001; McCormick et al., 2005; Neetoo et al., 2008; Scannell et al., 2000), pediocin PA-1/AcH (Ming et al., 1997; Santiago-silva et al., 2009), and enterocin (Iseppi et al., 2008; La Storia et al., 2012). Among these bacteriocins, nisin and pediocin PA-1/AcH are the only bacteriocins, to date, that have been approved for use in food. Although anti-listeria efficiency of nisin and pediocin significantly differed depending on the producing or indicator strains, the sample preparation method, and the bacteriocin assay conditions, pediocin is likely to have higher activity and acts more specifically against *L. monocytogenes* than nisin (Cintas et al., 1998; Rodriguez et al., 2002). In addition, pediocin PA-1, in contrast to both nisins A and Z, has potential to inhibit *Listeria* without disturbing other bacteria including beneficial ones (Blay et al., 2007).

Due to a trend toward active and green packaging, the use of biomaterials including cellulose, starch, pectin and poly(lactic acid) (PLA) have been more emphasized (Kuorwel et al., 2011; Liu et al., 2009; Rodriguez et al., 2006; Theinsathid et al., 2012). PLA is recognized as compostable biopolymer that attracts the interest for the packaging industry because of its outstanding properties and earth-friendly biodegradability. PLA exhibits many properties that are equivalent to or

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better than many petroleum-based plastics (Liu et al., 2009). Importantly, PLA packaging can be produced by many manufacturing processes, such as film blowing, injection molding, sheet extrusion, blow molding and thermoforming (Imam et al., 2008; Jamshidian et al., 2010). The combination of biodegradability of PLA with antimicrobial property of pediocin against a wide broad spectrum of food pathogen will be of full benefit as the active packaging. As a consequence, health-risk of consumers can be reduced. Shelf-life can be extended, thereby lowering the economic loss. Importantly, the waste of post use of this packaging will be decomposed through compostable system without causing environmental waste problems.

However, direct incorporation of antimicrobial peptide to PLA film has been limited by the hydrophobic characteristics of PLA. The incompatibility of bacteriocin in hydrophobic polymers caused the phase separation in film, leading to poor antimicrobial activity and mechanical properties. To solve this problem, sawdust particle (SP), a low-water solubility hydrophilic particle, was incorporated in PLA film to enhance adsorption of pediocin using diffusion coating technique. In contrast to the large amount of information on the antimicrobial activity of packaging films containing antimicrobials, to our knowledge no information is available about using natural fiber as carrier of pediocin PA-1/AcH in PLA film. Research on the possibility of using sawdust particle would lead to an alternative natural preservation method, easily applicable and of low cost. In addition, effect of pre-conditioning methods was also investigated to enhance the pediocin adsorption. Finally, anti-listeria activity of the PLA/SP biocomposite film toward a model pork system was determined in order to ensure the potential use of the film in real food system.

2. Materials and methods

2.1. PLA, saw dust, pediocin and other chemicals

PLA polymer 4042D was purchased from NatureWorks®. To prepare sawdust particle, wood sawdust was subjected to a cutter-mill (Wonder Blender, WB-1, Waring Products, Inc., Connecticut, USA) to obtain particles in the range of 100–300 μm and dried at 70 °C for 24 h in air-circulating oven before further pretreatment. Dried wood sawdust was stirred with absolute ethanol at room temperature for 1 h in order to eliminate the impurities on the surface of sawdust. Treated sawdust was dried at 70 °C for 3 h to evaporate the solvent and then soaked into 10% sodium hydroxide at 40 °C for 3 h to remove natural impurities including pectin, lignin, waxy substances and natural oils. Finally, processed wood sawdust was rinsed with distilled water to remove sodium hydroxide and dried at 60 °C for 24 h. The obtained powder was referred to as sawdust particle or SP.

Partially purified pediocin from *Pediococcus pentosaceus* BCC3772 (Kingcha et al., 2012) was prepared by the method of Zendo et al. (2003). *P. pentosaceus* BCC3772 was obtained from BIOTEC culture collection (BCC), National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand. Culture was maintained as frozen stock held in −80 °C in culture broth containing 15% (w/v) glycerol. Prior to use, culture was streaked on medium agar (1.5% casein sodium salt, 1.5% yeast extract and 1.0% glucose) and grown at 30 °C for 24 h. A single colony obtained from the plate was grown twice in culture broth and incubated at 30 °C for 24 h without shaking. The cell-free supernatant was obtained by centrifugation at 7500 ×*g* for 15 min at 4 °C using a Hi-speed centrifuge (Beckman: Avanti J-E). The anti-listeria substance was concentrated at 1000 ml of cell-free supernatant by hydrophobic interaction chromatography using an Amberlite XAD-16 polymeric resin (Sigma, USA). In brief, a 20 g of the resin was activated in 50% (v/v) isopropanol at 4 °C for 24 h. Thereafter, isopropanol was completely removed from the resin by washing with one volume of deionized water. The activated resin was added into a 1000 ml of the culture supernatant with gently mixed and kept at 4 °C for 24 h. The resin was loaded into the Econo fast flow column 2.5 × 30 cm (Bio-rad, USA) and washed with ss ml of deionized water, followed by 100 ml of 40% (v/v) ethanol in deionized water. The anti-listeria substance was eluted with 100 ml of 70% (v/v) isopropanol in deionized water containing 0.1% (v/v) trifluoroacetic acid (TFA). The eluent was then evaporated to get rid of isopropanol using rotary evaporator (40 °C for 30 min) and then freeze dried at −60 °C, 0.1 mBar and kept at 4 °C prior to use. The powder was referred to as partially purified pediocin.

2.2. Elaboration of PLA/SP biocomposite film

PLA/SP biocomposite film was fabricated using a blown film extrusion. Firstly, 5% (w/w) sawdust was mixed with PLA resin using treated sawdust, and subjected to a twin screw extruder (SHJ-36 twin screw extruder product line, Nanjing Chengke Machinery, China). Then, the composite film was fabricated via blown film technique. For PLA film without SP, the PLA resin was subjected to a blown film extruder to fabricate the control film without pre-compounding with SP. The thickness of the film was adjusted to 400–500 μm by controlling the take up speed.

2.3. Film pretreatment and coating procedure

PLA/SP biocomposite films were pretreated with three methods including dry-heat treatment (heating the film samples at 90 °C for 2 h in air-circulating oven), dry-heat treatment followed by acid treatment (soaking in 2% acetic acid for 30 min) and moist-heat treatment (heating the film samples at 90 °C, 90% RH for 2 h). Partially purified pediocin was loaded into pretreated films by a diffusion coating method according to Jin et al. (2009) and Liu et al. (2007) with some modifications. Briefly, eight (2 × 2 cm^2) film samples were soaked in a beaker containing 5 ml of 0.2% (w/v) partially purified pediocin solution for 30 min at room temperature. After pediocin adsorbed onto the film surface, the films were removed from the pediocin solution and washed three times with 5 ml of deionized water by shaking in the solution for 1 min for each time in order to eliminate the un-adsorbed pediocin. The washed films were dried under laminar flow for 30–60 min and stored at 4 ± 2 °C in a refrigerator prior to bacterial inhibition tests. To quantitatively determine the pediocin adsorption on composite film, the amount of pediocin retained in the solution was analyzed as the total protein content according to the method of Lowry et al. (1951). Pediocin adsorption on the film was calculated by the following equation:

$$\text{Pediocin adsorbed}\left(\mu g/cm^2\right) = [P1–P2]/A$$

where *P1* and *P2* represent the total protein (μg) of pediocin solution before and after adsorption, respectively and *A* represents the total surface area (cm^2) of the films used in the experiment.

2.4. Anti-listeria activity of film

Anti-listeria activity of film was evaluated using *L. monocytogenes* ATCC19115 as the indicator strain. Culture was maintained as frozen stock held at −80 °C in tryptic soy broth (TSB, Merck, Germany) containing 15% (w/v) glycerol. The indicator was streaked on TSB with 1.5% agar and incubated at 37 °C for 24 h. A single colony obtained on TSB agar was grown twice in TSB broth and incubated at 37 °C for 24 h. The inhibition of *L. monocytogenes* was evaluated using an agar diffusion method as described by Appendini and Hotchkiss (2002) and Jin and Zhang (2008) with some modifications. Film sample (2 × 2 cm^2) was placed on the surface of TSB agar plate. The plate was overlaid with 5.0 ml of the semi-soft TSB agar (1.0% (w/v) agar) containing approximately 10^6 CFU/ml of *L. monocytogenes* ATCC19115. After the plates were incubated at