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Control of *Listeria monocytogenes* on ham steaks by antimicrobials incorporated into chitosan-coated plastic films

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Abstract

Contamination of ready-to-eat (RTE) meat products such as ham steaks with *Listeria monocytogenes* has been a concern for the meat processing industry. The objective of this study was to evaluate the antilisterial efficacy of chitosan-coated plastic films alone or incorporating five generally recognized as safe (GRAS) antimicrobials. Effect of chitosan-coated plastic film on the growth of *L. monocytogenes* was first investigated in an aqueous system of culture medium broth and chitosan-coated films were able to inhibit the growth of *L. monocytogenes* in a concentration-dependent manner. However, chitosan-coated plastic films were not able to control the growth of *L. monocytogenes* on ham steaks. Therefore, five GRAS antimicrobials were subsequently incorporated into chitosan-coated plastic films to enhance their antilisterial effectiveness. Ham steaks were surface-inoculated with a five-strain cocktail of *L. monocytogenes* and then packaged in chitosan-coated plastic films containing 500 IU/cm^2 of nisin, 0.01 g/cm^2 of sodium lactate (SL), 0.0025 g/cm^2 of sodium diacetate, 0.003 g/cm^2 of potassium sorbate (PB), or 0.001 g/cm^2 of sodium benzoate (SB). The samples were stored at room temperature (ca. 20 °C) for 10 days. Incorporating antimicrobials into chitosan-coated plastic films slowed down or inhibited the growth of *L. monocytogenes*. The chitosan-coated plastic film containing SL was the most effective antimicrobial film and its efficacy against *L. monocytogenes* on ham steaks was evaluated during 12-week storage at 4 °C. The film showed excellent long-term antilisterial effect with the counts of *L. monocytogenes* being slightly lower than the initial inoculum. Chitosan-coated plastic films containing 0.001 g/cm^2 of SL have a potential to be used on ham steaks to control *L. monocytogenes*.
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Keywords: Chitosan; *Listeria monocytogenes*; Ham steaks; Antimicrobial packaging; Antimicrobials; Nisin; Sodium lactate; Sodium diacetate; Potassium sorbate; Sodium benzoate

1. Introduction

Listeria monocytogenes is a foodborne pathogen of particular concern in ready-to-eat (RTE) meat products because of its ability to survive and grow at refrigeration temperatures, its capacity to tolerate relatively high heat and high concentrations of salt and the high fatality rate associated with listeriosis. *L. monocytogenes* has been involved in numerous foodborne illness outbreaks associated with RTE meats. In 1998 and 1999, a significant outbreak occurred with frankfurters, which resulted in 21 deaths and approximately 100 reported cases of listeriosis (Centers for Disease Control and Prevention (CDC), 1999). Another notable outbreak occurred in the northeastern United States in 2002, resulting in 10 fatalities associated with the consumption of sliced turkey deli meat (CDC, 2000, 2002). *L. monocytogenes* accounts for 28% of the deaths resulting from foodborne illnesses in the US, which is second only to *Salmonella* (31%) (Mead et al., 1999).

L. monocytogenes is a frequent surface contaminant of RTE meats often occurring during the post-processing phase (Tompkin, 2002). Novel means to control this contamination have been sought (Wakabayashi et al., 1992). Antimicrobial packaging can be a promising tool for protecting RTE meats from *L. monocytogenes* contamination (Janes et al., 2002; Lungu and Johnson, 2005). Antimicrobial packaging films act by preventing microbial growth on a food surface by direct contact of the package with the surface of food. The gradual release of an

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antimicrobial substance from a packaging film to the food surface for extended period of time may be more advantageous than incorporating the antimicrobial into foods. In the latter processes, antimicrobial activity may be lost or reduced due to inactivation of the antimicrobial compound by food components (Appendini and Hotchkiss, 2002; Quintavalla and Vicini, 2002).

Chitosan is a natural polymer obtained by deacetylation of chitin, which is the major constituent of the exoskeleton of crustaceans. Chitosan has been proved to be nontoxic, biodegradable, and biocompatible. Chitosan is insoluble in water, but soluble in various acidic solvents such as dilute hydrochloric, formic and acetic acids. In an acidic solution, the amine groups on the chitosan molecule are protonated to NH^{3+} and thus acquire a positive charge (Shahidi et al., 1999; Ravi Kumar, 2000). Chitosan has intrinsic antimicrobial activity and inhibits the growth of a wide variety of bacteria (Shahidi et al., 1999; Helander et al., 2001). In this study chitosan was used as a carrier for antimicrobials. Since edible film formed by chitosan is brittle and does not have good mechanical properties, in this study chitosan was coated onto a plastic film to overcome these shortcomings. The additional benefit of coating chitosan onto plastic films is that chitosan is not consumed with food. Using edible coatings to carry functional substances is not a new concept. Plastic films coated with edible coatings carrying spices and flavoring substances are commercially used to transfer those substances to the surfaces of meat, poultry and fish products.

To enhance the efficacy of chitosan-coated film against *L. monocytogenes*, five generally recognized as safe (GRAS) antimicrobials, nisin, sodium lactate (SL), sodium diacetate (SD), potassium sorbate (PB), and sodium benzoate (SB), were incorporated into the chitosan coating in this study. It has been well documented that these antimicrobials can control *L. monocytogenes* in meat products (Samelis et al., 2002; Szabo and Cahill, 1999; Glass et al., 2002; Schlyter et al., 1993; Lu et al., 2005; Islam et al., 2002). Nisin, a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, exerts rapid bactericidal effects against Gram-positive bacteria, especially against strains of *L. monocytogenes*, in laboratory media or model food systems (Delves-Broughton and Gasson, 1994). SL is primarily used as a flavor enhancer in meat and poultry products (Shelef, 1994). SD is a derivative of acetic acid and is used in bread and cakes to prevent mold growth (Jay, 2000). At 0.1–0.3%, SD can control growth of *L. monocytogenes* in meat (Schlyter et al., 1993; Ghanem and Skonberg, 2002). PS is primarily used to control yeasts and molds. Effective antimicrobial concentrations of PS in most foods are in the range of 0.05–0.30% (Sofos and Busta, 1993). El-Shenawy and Marth (1988) found that PS inhibited or inactivated *L. monocytogenes* in a broth substrate, depending on pH and concentration. The antibacterial properties of SB are due to the undissociated, molecular form of benzoic acid (Doores, 1993). These studies have investigated the efficacy of these antimicrobials when directly added into or onto food products. An alternative way of controlling *L. monocytogenes* is through their incorporation into a packaging material that is subsequently applied onto food. To our knowledge, no research has been conducted to compare the effectiveness of these five antimicrobials incorporated into packaging films for controlling *L. monocytogenes*.

The objectives of this study were to evaluate the efficacy of chitosan-coated plastic films and chitosan-coated plastic films incorporating antimicrobials on controlling the growth of *L. monocytogenes* on ham steaks. The effectiveness of these antimicrobial packaging films was evaluated at room temperature. The most effective antimicrobial film was selected and its efficacy against *L. monocytogenes* on ham steaks was assessed at refrigeration temperature (4 °C) for 12 weeks.

2. Materials and methods

2.1. Effect of chitosan-coated plastic film on the growth of *L. monocytogenes* in a culture medium broth

2.1.1. Coating of plastic film with chitosan

Two grams of medium molecular weight (MMW) chitosan (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 100 ml of 1% (w/v) acetic acid and stirred overnight at room temperature (chitosan concentration = 0.02 g/ml or 2%). Hydroxypropyl methylcellulose (HPMC) (Sigma-Aldrich) solution was prepared by dissolving 3 g of HPMC and 0.33 ml of polyethylene glycol 400 (Fisher Scientific, Hampton, NH, USA) in 100 ml of 1% acetic acid. The coating solution of the first layer was prepared by mixing equal volumes of the chitosan and HPMC solutions according to the method described by Moller et al. (2004).

A Surlyn® film (2.0 mil) was taped to $20 \times 20\,cm^2$ glass plates and 15 ml of the chitosan–HPMC coating solution was cast onto the plastic film using a thin-layer chromatography plate coater (TLC, CAMAG, Muttenz, Switzerland). The gate of the TLC coater was fixed at 500 μm to control the thickness of the coating. The coated film was air-dried at room temperature overnight. Then 15 ml of the chitosan solution was cast onto the first layer of coating and air-dried overnight to form a second layer and the same procedure was repeated for the third layer using the same volume of chitosan solution. It was found that without incorporating HPMC, the first chitosan layer could not be coated uniformly onto the plastic film. The chitosan-coated film contained 0.0025 g of chitosan per cm^2 of film surface. A control film was made by coating a Surlyn® film with the HPMC solution. The films were subsequently cut into discs with diameter of 1.2 cm and UV-treated for 2 min to sterilize the films.

2.1.2. Indicator microorganism

L. monocytogenes ATCC 19115 was maintained on tryptic soy agar plus 0.6% yeast extract (TSAYE) (Difco Laboratories, Detroit, MI, USA) agar plates at 4 °C. The

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