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Application of a novel antimicrobial coating on roast beef for inactivation and inhibition of *Listeria monocytogenes* during storage

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ABSTRACT

The antilisterial efficacy of novel coating solutions made with organic acids, lauric arginate ester, and chitosan was evaluated in a three-stage study on inoculated roast beef for the first time. Ready-to-eat roast beef was specially ordered from the manufacturer. The meat surface was inoculated with five-strain *Listeria monocytogenes* cocktail inoculums at two different levels, ~3 and 6 Log CFU/cm^2 and treated with the stock solution (HAMS), the 1:5 diluted solution (MAMS), and the 1:10 diluted solution (LAMS) (stage 1). During the 20 min contact time, the antimicrobial coatings reduced the *Listeria* populations by approximately 0.9–0.3 Log CFU/cm^2. The higher the concentrations of the antimicrobial solution, the better the antilisterial effects were. The treated inoculated beef samples were then stored at 4 °C for 30 days. During storage, *Listeria* growth inhibition effects were seen. While no growth was seen from the HAMS-treated samples, a 1.6 Log CFU/cm^2 increase was seen for MAMS-treated samples, a 4.6 Log CFU/cm^2 increase was seen for LAMS-treated samples, and a 5.7 Log CFU/cm^2 increase was seen for NoAMS-treated samples on Day 30 (~3 Log CFU/cm^2 inoculation level). In the second stage, the impact of the roast beef storage time on solution's antilisterial effect was evaluated. Results showed that the effect of the antimicrobial solution was dependent on both the initial inoculation levels and storage times. In stage 3, the effect of the antimicrobial solution on roast beef quality was studied with both instrument measurement and sensory evaluation. Minor changes in color, pH, and water activity were found. However, only limited sensory differences were seen between the treated and untreated samples. When panels were able to accurately find color differences between samples, they preferred the treated samples. The findings of this research proved the antilisterial efficacy of the novel antimicrobial solution and showed its potential for being used as a roast beef cut surface coating to control *Listeria* contamination and for color protection.

1. Introduction

The post-lethality contamination of ready-to-eat (RTE) meat with *Listeria monocytogenes* has been a significant public health concern. According to the 2003 risk assessment by the U.S. Food and Drug Administration in cooperation with the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS), "delicatessen meats were identified as possessing the highest risk among the 23 categories of RTE foods" (FDA-CFSAN, 2003; Zhang et al., 2012). The lack of heating immediately prior to consumption elevates the risk for listeriosis; if an initial contamination by *L. monocytogenes* happens, no further inactivation steps are usually involved. Several studies have pointed to the post-processing manipulations as important factors for the presence of *L. monocytogenes* (Aguado et al., 2001; Glass and Doyle, 1989; Rørvik et al., 1995); the retail site has been identified to be one of the important places for cross-contamination. In a large-scale survey of RTE foods collected from retail markets in the U.S., Gombas et al. (2003) found that in-store-packaged deli meats were more likely to harbor *L. monocytogenes* than were manufacturer-packaged products. Cross-contaminations between the processing equipment and deli meats have been demonstrated by multiple studies (Keskinen et al., 2008a,b; Lin et al., 2006).

To inhibit the proliferation of *L. monocytogenes* and other foodborne pathogens, antimicrobials, such as lactate and diacetate, have been added to RTE meats during processing (Zhang et al., 2012). The ability of *L. monocytogenes* to grow in deli meats varies based on the product formulation, storage temperature, and the presence of native microflora (Beumer et al., 1996; Glass and Doyle, 1989; Hwang and Sheen, 2010; Lianou et al., 2007; Zhang et al., 2012). Zhang et al. (2012) evaluated the growth of *Listeria* on four types of deli meats (with or without lactate or diacetate) under different aerobic consumer storage conditions. Their results showed that although lactate and diacetate suppressed *Listeria* growth, the extent of inhibition differed, ranging from total

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inhibition in roast beef to only partial inhibition in ham and cured turkey. Considerable variations of antimicrobial concentrations were also seen in that study when different production lots of the same product were analyzed. For example, the concentration of acetate in brand G cured turkey ranged from 0.32 to 1.12 mg/g with an average of 0.62 mg/g, resulting in variations in the *Listeria* growth (Zhang et al., 2012).

Given the facts that concentrations of antimicrobials in RTE meats differ from lot to lot and the deli counter is one of the critical contamination/cross-contamination sites, new techniques that can prevent *Listeria* contamination and inhibit the *Listeria* growth during the deli counter or consumer home processing/storage are needed. In the last decade, researchers evaluated various means of improving the safety of deli meat products, including using antimicrobial packaging, films, or coatings (Eswaranandam et al., 2006; Grower et al., 2004; Guo et al., 2014a; Janes et al., 2002; Jin et al., 2009a,b; Jin and Zhang, 2008; Lungu and Johnson, 2005; Min et al., 2010), applying antimicrobial treatments such as organic acids (Samelis et al., 2001, 2005) and bacteriocin (Samelis et al., 2005) prior to final packaging, or employing post-packaging pasteurization such as high pressure and thermal treatments (Aymerich et al., 2005; McCormick et al., 2005). These studies evaluated the efficacy of different antimicrobial solutions and application methods. However, limited research has investigated the impacts of antimicrobial solutions or treatments on product quality and even less research has taken the influence of meat shelf life (storage time) into consideration. In addition, there is limited information on the antimicrobial effect of multiple organic acids in combination with LAE in stock solutions or in diluted solutions.

The objective of this study was to evaluate a new antimicrobial coating for direct application onto deli meat cut surfaces in counter delis, to prevent cross-contamination and inhibit the growth of *Listeria* at the cut face, and to minimize the chance of secondary transfer of *Listeria* from the cut face to the slicer. The proposed antimicrobial solution is made with generally recognized as safe (GRAS) ingredients. The key ingredients include Lauric arginate ester (LAE), chitosan, and organic acids. LAE has been verified to be nontoxic after consumption (Ruckman et al., 2004) and its effective antimicrobial functions and mechanisms have been studied by Rodriguez (2004). Chitosan is added to the antimicrobial solution for its excellent film-forming properties as well as its antimicrobial properties. The chitosan in the solution allows the solution to better coat the meat surface (Guo et al., 2014a).

To fully evaluate the potential use of this antimicrobial coating on RTE roast beef, the three specific objectives of this study were: 1, determine the solution's most efficient antilisterial concentrations by using roast beef as a model; 2, evaluate the impact of roast beef storage time on the solution's antimicrobial effect; and 3, investigate the potential influence this antimicrobial solution has on roast beef quality by using instrument measurement and sensory panel evaluation.

2. Materials and methods

2.1. Antimicrobial solution preparation

The stock antimicrobial solution was made first by mixing 5% chitosan in an acid solution containing 2% each of acetic, lactic and levulinic acids. Lauric arginate ester (LAE, 20% stock solution) was then added to the chitosan-acid solution at 200 μl/ml, as described in our previous study (Guo et al., 2014a). The mixture was stirred overnight until the polymer was completely dissolved. All the chemicals used were purchased from Sigma-Aldrich (St. Louis, MO, USA), except the LAE, which was purchased from A&B Ingredients (Fairfield, NJ, USA). The stock solution was kept in the refrigerator until used. The antimicrobial efficiency of three concentrations was examined; they were the stock solution (HAMS), the 1:5 dilution (MAMS), and the 1:10 dilution (LAMS). Autoclaved MilliQ water was used as the control (NoAMS).

2.2. Bacterial strains and inoculum preparation

A five-strain *L. monocytogenes* cocktail was used in this study, including *L. monocytogenes* Scott A (serotype 4b, ATCC 49594, a clinical isolate from a New England outbreak), *L. monocytogenes* 101A (serotype 4b, a beef and pork sausage outbreak isolate), *L. monocytogenes* 108 M (serotype 1/2b, a salami isolate), *L. monocytogenes* ATCC 19115 (serotype 4b, a clinical isolate), and *L. monocytogenes* ATCC 7644 (serotype 1/2c, a clinical isolate). These strains were stored at −80 °C in the food microbiology lab located on the Auburn University campus.

To prepare the inoculum, overnight pure broth cultures (in 10 ml Brain-Heart Infusion Broth) were washed by centrifugation at 3500 rpm for 10 min at 4 °C. Each culture was then resuspended in 10 ml of autoclaved 1× phosphate buffered saline (PBS). All bacterial media used in this study were purchased from Difco, Becton Dickinson (Sparks, MD, USA) unless otherwise stated. The OD_{600} values of each culture were adjusted to ~1.0 by PBS and equal volume of every culture suspension was mixed together to form the stock cocktail. The stock cocktail was used directly for inoculating samples designated for the "high inoculation level" study (~6 Log CFU/cm^2) and the diluted stock cocktail was used for inoculating samples designated for the "low inoculation level" study (~3 Log CFU/cm^2).

2.3. Meat sample preparation

Roast beef used in this study was ordered directly from the manufacturer's plant and shipped immediately from the production line to the lab. For the stage 1 and 2 studies, fully-cooked medium well choice roast beef (cap off, top round, whole) was used. For the stage 3 study, both fully-cooked medium well and rare choice roast beef (cap off, top round, whole) were used. They were kept at −20 °C until used. One day before the roast beef was sliced and inoculated, the roasts were taken out of the −20 °C freezer and thawed at 4 °C for 24 h. All roast beef products used were proven to be *L. monocytogenes* free following the FDA Bacteriological Analytical Manual (BAM) (FDA, 2011).

2.4. Determination of effective antimicrobial concentrations (stage 1)

Medium well roast beef was sliced into 2.0 mm slices and then cut into 8.57 × 8.57 cm^2. Two hundred microliters of stock or diluted culture cocktail was evenly spread onto one side of each piece of roast beef (two inoculation levels were achieved: ~3 Log CFU/cm^2 and ~6 Log CFU/cm^2). The inoculated roast beef was kept under the biosafety hood for 20 min to allow *Listeria* cells to attach. After the 20 min cell attachment time, 900 μl of antimicrobial solution (stock or diluted) or 900 μl of MilliQ water (NoAMS) was applied to the inoculated side of the roast beef as a coating, followed by 20 min contact time (time 0). Two slices of inoculated and antimicrobial solution-treated roast beef were placed in every Whirl-Pak bag (Nasco, St. Charles, MO, USA) and stored in the refrigerator at 4 °C for 30 days. Three bags of samples were taken every 5 days to enumerate *Listeria* cells.

To enumerate *Listeria* cells on roast beef, 100 ml 0.1% buffered peptone water was added to each sample bag and homogenized for 2 min in a smasher (AES Chemunex, bioMérieux, France) and two 100-μl samples of the homogenates or their dilutions were taken and spread onto two modified Oxford agar plates (with supplements) (Difco, Becton Dickinson) respectively. Plates were incubated at 35 °C for 48 h and *Listeria* colonies were counted following the manufacturer's instruction. The detection limit of the direct plating method was 1.8 Log CFU/cm^2. After plating the samples, 125 ml of 2× buffered *Listeria* enrichment broth was added to each bag and the samples were enriched at 30 °C for 48 h before the enriched suspension was streaked onto Oxford agar. The plates were checked for presence/absence of *Listeria* colonies after additional 48 h incubation. Suspect *Listeria* colonies were picked and confirmed with polymerase chain