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Product safety

POLYLACTIC ACID AS A DECONTAMINATION AGENT FOR BEEF CARCASSES

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ABSTRACT

Decontamination of beef carcasses with low molecular weight (LMW) polylactic acid (PLA) and the combined effects of decontamination and vacuum packaging on reduction of microbial numbers were studied. In Part 1, carcass surfaces were contaminated with a manure inoculum and either PLA (2.5%, 40°C) or water was sprayed on as a decontamination method. Aerobic (APC) and Enterobacteriaceae (EC) were determined immediately and after 8 days of storage (4°C). PLA reduced the mean APC from sirloins by 2.05 log cfu/cm^2 and from plates by 2.49 log cfu/cm^2 ($p<0.001$) compared to initial APC of 5.20 log cfu/cm^2. EC was reduced 3.55 log cfu/cm^2 and 3.98 log cfu/cm^2 for sirloins and plates, respectively. After day 8, microbial numbers were not significantly increased. In Part 2, strips (10X40 cm) were removed from hot carcasses after decontamination and vacuum packaged for up to 8 weeks at 4 C. Mean pH values decreased from 6.1 to 4.8 after PLA treatment. Initial APC and EC were reduced by 3.39 or 4.64 log cfu/cm^2, respectively, for PLA ($p<0.001$). APC and EC increased, but remained less than control samples, at 2 weeks but all counts were greater than initial contamination values for 4 and 8 weeks of storage. PLA is effective in lowering initial microbial contamination of beef carcasses.

INTRODUCTION

In 1993, the tragic food-borne outbreak of *E. coli* O157:H7 in the United States has received tremendous attention. Since then, researchers have developed new effective chemical methods and advanced techniques to minimize the pathogenic and spoilage bacteria on animal carcasses. Carcass washing with organic acid solutions or hot water is one method used currently to decontaminate microbial contamination and is a part of Hazard Analysis and Critical Control Point (HACCP)-based program to reduce pathogenic and spoilage bacteria on retail beef cuts.

Lactic acid decontamination is one method used to sanitize fresh meat carcasses and retail cuts (Anderson and Marshall,1990; Anderson et al, 1992). Its acidity affects respiring microbial cells by affecting the functions of bacterial enzymes and transport of nutrients into the cells. The pH drop and the periods of maintaining an effective pH depend upon the amount of lactic acid, the rate of diffusion into the meat, and meat buffering. To extend the shelf-life and to minimize pathogens on fresh beef, an effective pH should be maintained for a long period of time.

Recently, a group of researchers in University of Missouri-Columbia has developed and conducted studies with low molecular weight (LMW) polylactic acid (PLA). Low molecular weight polylactic acid continuously releases free lactic acid and a low pH level can be maintained for a longer period than that of conventional free lactic acid. Early testing has shown that PLA has a prolonged antimicrobial activity both in broth culture and on beef surfaces (Jin, 1995). Before being approved by the United States Department of Agriculture (USDA) - Food Safety and Inspection Service (FSIS) and the Food and Drug Administration (FDA), more reliable data supporting the efficacy of low molecular weight polylactic acid as an antimicrobial agent are required.

The purposes of the present study were to i) evaluate the immediate bactericidal effect of low molecular weight polylactic acid on the beef carcasses after carcass washing and ii) to investigate the delayed effects of the decontamination of beef cuts in vacuum packages.

MATERIALS AND METHODS

Preparation of manure inocula. The inocula were prepared by mixing 50 g of fresh manure with 150 ml of sterile butterfield buffered solution (K_2HPO_4 4g and KH_2PO_4 8 g in 1 L distilled water) in a stomacher bag and stomaching for 2 min. The mixture was filtered with sterile cheese-cloth and the filtrate was stored at 23°C overnight for use the next morning. Aerobic bacteria and *Enterobacteriaceae* in the inocula were tested on the experimental days.

Part I. The experiment was a 2 x 2 x 3 factorial in a split plot design with four replications. The conditions were two treatments (2.5% PLA & hot water), two locations (sirloin & plate), and three sampling times (before contamination, after treatments and 8th day).

Each slaughter day, a dairy cow was slaughtered with standard procedures including captive bolt stunning, exanguination, manual dehiding, evisceration and thorough water. Five minutes after water showering, two carcass surface regions (sirloin and plate) were contaminated with inoculum of manure and air dried for 5 min. Before decontamination with a spray washing technique, four core samples from the carcass surface of the sirloin and plate were randomly sampled. Each core (2.54 cm diameter, 2 mm approximate depth) was placed in a separate stomacher bag and stored in an ice box before transfer to a laboratory. The entire surface of the left side was sprayed with 2.5% (v/v) polylactic acid solution (40°C, pH 2.5) by using a custom-made automatic sprayer for 60 s. The solution was sprayed at a pressure of 300 psi, flow rate of 80 L/min, application rate of 0.127 m /sec and a distance from the rail of 0.279 m. The right side was sprayed with hot water(40°C) as control. Four cores from the previous locations were removed at 5 min after decontamination. The carcasses were moved immediately into the carcass chilling room (4°C) and after hanging for a week, four cores of each region were collected.

Part II. The experiment was a 2 x 5 factorial in a randomized block design with four replications.

This experiment followed the procedure in part I. After contamination and decontamination of the carcass surface, four core samples were collected for bacterial counts. Strips (10 x 40 cm) were removed from hot carcasses and vacuum packaged and stored at 4°C for up to 8 weeks. At 2, 4 and 8 weeks after decontamination, four core samples were sampled and determined for APC and Enterobacteriaceae. The surface pH of the carcass and strips was determined before and after treatment, and 2, 4, or 8 weeks

Bacteriological examination. Each core sample was stomached with 99 ml of 0.1% peptone water solution (Difco) in a stomacher for 2 min and was diluted in 0.1% peptone water solution for three plating dilutions. A pour plate technique was used for microbial counts. Aerobic microorganisms (APC) were enumerated in Tryptone Glucose Yeast Agar (Difco). For Enterobacteriaceae count (EC), the samples were plated on TSA (Difco) to recover the stressed organisms and then overlayed with an equal amount of VRBGA-2 (Difco) after 15 min. APC and EC were incubated at 32°C for 48 h and 24 h, respectively (Anderson and Marshall, 1990). Bacterial counts were expressed as colony-forming units (CFU) per cm^2 and then converted to $\log_{10}$ ($\log_{10}$ cfu/cm^2).

Statistical analysis. Data were analyzed by General Linear Model (GLM) procedure of the Statistical Analysis System (SAS). Difference in mean log CFU/cm^2 among treatments or storage periods were determined by using least-squares methods.

RESULTS & DISCUSSION

APC and EC in the manure were 8.37 log cfu/cm^2 and 8.04 log cfu/cm^2 in Part I, and 7.95 log cfu/cm^2 and 7.05 log cfu/cm^2 in Part II.

In Part I, APC and EC were determined immediately and after 8 days of storage at 4°C (Figure 1.). The immediate bactericidal effect of 2.5% PLA was presented by reducing the mean APC from sirloins by 2.05 log cfu/cm^2 and from plates by 2.49 log cfu/cm^2 ($p<0.001$) compared to initial APC of 5.20 log cfu/cm^2. EC was reduced from 4.75 log cfu/cm^2 by 3.55 log cfu/cm^2 and 3.98 log cfu/cm^2 for sirloins and plates, respectively ($p<0.001$). Analysis of variance showed that only main effect of treatment influenced reducing the amount of bacteria ($P<0.001$). The samples collected from plates or sirloins did not affect to the number of bacteria counts. There were interaction effects between treatment and sampling times ($p<0.01$). At day 8 after decontamination, the microbial numbers of plates and sirloins were not significantly increased (3.0 log cfu /cm^2 & 2.66 log cfu/cm^2). These results indicated the sustained bactericidal effect of PLA. However, moisture on the carcass surfaces may be another factor inhibiting bacterial growth because the numbers of bacterial counts on carcass surfaces treated with water rinsing did not increase significantly at day 8 after decontamination(4.2-4.4 log cfu/cm^2 & 4.0-4.6 log cfu/cm^2). The carcass surfaces were dried after chilled in a cool room for a week. Water rinsing could reduce the amount of bacteria by approximately 1.0 log cfu/cm^2 but its efficacy was much lower than 2.5% PLA ($p<0.01$).

In Part II, the mean surface pH values of the strips stored at 4°C under vacuum-packaging for up to 8 weeks decreased from 6.1 to 4.8 after PLA treatment and maintained at 4.9 after 2 week decontamination. The surface pH increased to 5.1 and 5.2 at week 4 and 8, respectively. The result suggested meat buffering at a low rate. The surface pH of control was unchanged after treatment (6.1 to 5.9) but slightly declined to 5.6, 5.4 and 5.2 at 2, 4 and 8 weeks after storage, respectively.

The effect of 2.5% PLA on the reduction of APC and EC after up to 8 weeks of storage at 4°C was presented in figure 2. The initial APC and EC (5.36 & 4.83 log cfu/cm^2) were reduced by 3.39 or 4.64 log cfu /cm^2, respectively ($p<0.001$). At 2 weeks after decontamination, the counts of samples treated with 2.5% PLA under vacuum-packaging were increased from 1.97 log cfu/cm^2 to 3.31 log cfu/cm^2 for APC and 0.19 log cfu/cm^2 to 1.88 log cfu/cm^2 for EC ($p<0.001$) but remained less than control samples($p<0.01$). By 4

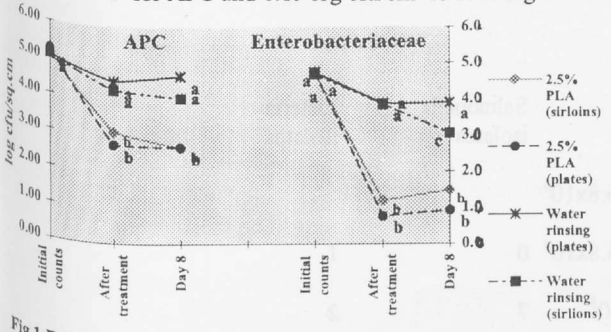

Fig 1 Effect of 2.5% PLA for APC and EC on beef carcasses stored at 4 C.

*Means with the same letter do not differ significantly among storage times at $p<0.001$.

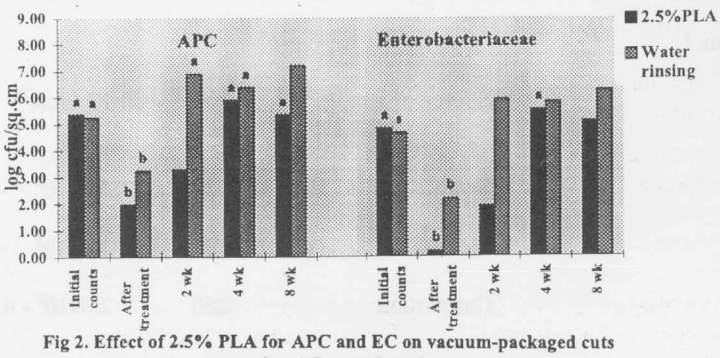

Fig 2. Effect of 2.5% PLA for APC and EC on vacuum-packaged cuts stored at 4 C up to 8 weeks.

*Means with the same letter do not differ significantly among storage times at $p<0.001$.

and 8 weeks of storage, all counts were slightly greater than initial contamination values(5.90 log cfu/cm^2 for APC & 5.54 log cfu/cm^2 for EC at 4 weeks and 5.35 log cfu/cm^2 for APC & 5.08 log cfu/cm^2 at 8 weeks, $p<0.001$). The counts of APC and EC of control samples were reduced approximately 2 log cfu/cm^2 but were greatly increased over the initial counts at 2 weeks of storage ($p<0.0.001$). Analysis of variance confirmed that treatment and storage periods affected change of the amounts of bacterial count and there was an interaction effect of treatment and storage times ($p<0.0001$). The counts of the samples treated with PLA increased and corresponded with the surface pH values of the samples. The useful pH which delays bacterial growth should be around 4.5. Lactic acid has reduced the numbers of bacteria on the meat surfaces up to 3 log cfu/cm^2 in some reports, while in this study the PLA showed more than 3 log cfu/cm^2 of Enterbacteriaceae could be reduced. This study showed that 2.5% of low molecular weight polylactic acid is more effective than water in lowering initial microbial contamination of beef carcasses. A study is underway in our laboratory which involves the application of 2% polylactic acid on beef steaks for purpose of reducing pathogenic and spoilage bacteria to pose a health risk of consumers and extend shelf-life of retail cuts.

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