PE5.03 Control of listeria monocytogenes on ready-to-eat (RTE) uncured turkey rolls by formulation with a "delayed-release" organic acid 281.00

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Abstract—Ready-to-eat (RTE) meat or poultry products have been implicated as a significant source of listeriosis infection in humans and U.S. regulations require that processors employ lethal or inhibitory antimicrobial strategies during production to control the growth of the pathogen potentially introduced after lethality processing. Use of a delayed-release organic acid rather than direct addition of organic acid in the emulsion (which would result in yield, loss, textural issues, etc.) combined with organic salt(s) was proposed for controlling Listeria monocytogenes. In this study, cooked uncured turkey rolls were formulated with 1.4% potassium lactate and 0.1% sodium diacetate (PLSD), 0.3% coated citric acid (CCA), and the combination of PLSD and CCA. After cooking, turkey rolls from each batch were chopped to make 600-g bulk portions that were inoculated (ca. 3 log CFU/g) with a composite of L. monocytogenes strains before vacuum packaging as individual 25-g samples and storage at 7ºC for up to 97 d. Changes in L. monocytogenes populations were evaluated at appropriate intervals during storage. Furthermore, the pH of the brine and water activity, moisture/loss on drying, yield (%), and pH of the final product after cooking was determined. Use of only CCA in the product formulation resulted in growth of L. monocytogenes to 8 log CFU/g by day-14. Product formulation with PLSD alone experienced outgrowth of L. monocytogenes to 8 log CFU/g by day-31. The combination of PLSD and CCA provided complete inhibition of L. monocytogenes growth and even a bactericidal effect (reduction of 0.7 log CFU/g) throughout storage. Combining a delayed-release organic acid, specifically coated citric acid, with a lactate/diacetate blend in the formulation inhibits the growth of L. monocytogenes on cooked uncured turkey rolls up to 97 d at refrigerated storage.

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Index Terms—RTE meat, uncured turkey roll, Listeria monocytogenes, coated organic acid, lactate, diacetate

I. INTRODUCTION

READY-TO-EAT (RTE) meat or poultry products contaminated with Listeria monocytogenes are implicated in several outbreaks of listeriosis in the United States annually [5]. In response to the frequency and magnitude of recalls and outbreaks associated with L. monocytogenes in RTE meat and poultry products, the USDA Food Safety and Inspection Service (USDA/FSIS) enforces a zero-tolerance rule for the pathogen's presence in these products as well as requiring processor's to apply control measures in the products if they are exposed to the processing environment after the lethality processing step and may support growth of the pathogen [7,8]. Specifically, USDA/FSIS requires processors to use one of three alternatives to control L. monocytogenes in RTE meats: (i) a postleathality inactivation treatment AND a L. monocytogenes growth inhibitor (least testing frequency); (ii) a postlethality inactivation treatment or a growth inhibitor (moderate testing frequency); or, (iii) sanitation measures (most frequent testing) [7]. The antilisterial effect of "generally recognized as safe" compounds, such as organic acids and their salts, employed as dipping solutions or as formulation ingredients have been used effectively in RTE meat products to meet the regulatory requirements [1,4,6]. Many combinations of various food antimicrobials are able to control L. monocytogenes in RTE meats, however, lactate and diacetate salts especially when used in combination appear to be particularly effective at controlling the pathogen in RTE meats held at refrigeration temperatures [5] and are indeed recommended by the USDA/FSIS for control of the pathogen. Uncured RTE meats are more sensitive to outgrowth of L. monocytogenes and as such need

higher levels of lactates/diacetates to control the pathogen; however this has a substantial taste impact. It was hypothesized that a slight pH reduction in the final product would significantly improve the effects of lactate/diacetate blends. Simulation of the antilisterial effect of a lactate/diacetate blend with lower final product pH using a predictive model [3] demonstrated that L. monocytogenes outgrowth could effectively be controlled during prolonged storage at refrigeration temperatures. The invention of coated organic acids that have controlled release into the immediate matrix provides a means of achieving effective meat acidification in the formulation. The authors conceived that inclusion of a delayed-release (latent) organic acid combined with organic acid salts in the formulation of RTE meat products would effectively control L. monocytogenes [2]. The objective of the current study was thus to test the antilisterial effect of a "delayed-release" organic acid, in the form of coated citric acid, when combined with a lactate/diacetate blend in the formulation of cooked, uncured turkey rolls.

II. MATERIALS AND METHODS

A. Preparation of inocula Listeria monocytogenes strains (NRRL B33028, NRRL B33039, NCTC 12480, NCIMB 13449, and LMG 23193) were activated individually in Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) broth (24 h at 30ºC) from frozen stock cultures. Activated cultures were transferred again into BHI and incubated for 24 h at 30ºC. Equivalent populations of each isolate were combined to provide a five-strain mixture of L. monocytoegenes to yield a target level of approximately 3 log CFU/g of final product per package.

B. Preparation and treatment of uncured turkey rolls The basic uncured turkey roll formulation (3.6 kg batch) consisted of ground turkey breast (80% w/w; approximately 1% fat), water (10%), ice (2.27%), sodium chloride (1.90%), sodium triphosphate (0.42%), maltodextrin (1.50%), carrageenan (0.70%), and wheat starch (3%). The basic formulation was modified to produce three formulation batches (each 1.2 kg) to contain: (i) 0.42% of a 72% (w/w) commercial product equivalent to 0.3% coated citric acid (CCA) (Karmat, Kibbutz Ramot Menashe, Israel); (ii) 2.5% (w/w) of a commercial blend of lactate/diacetate equivalent to 1.4% potassium lactate (PL) and 0.1% sodium diacetate (SD) (PURASAL Opti.Form PD4, PURAC, Inc., Lincolnshire, IL); and, (iii) the combination of CCA and PLSD (use levels as described above). The ground turkey meat (stored for 24 h at 0ºC before use) and all of the nonmeat ingredients (dissolved to form the brine) were processed for 4 min (maintaining temperature at below 13ºC) in a cutter/mixer (R10 V.V. Robot Coupe (de Jager, Dordrecht, the Netherlands) under vacuum. The batter was packed in bags and tumbled twice (2-h cycles with 30 min between each tumble cycle) and stored for 16 h at 0ºC for brine distribution. Rolls (mean diameter of 5.5 cm) were prepared by stuffing batter into cooking bags and cooked to an internal temperature of 72ºC in a water bath. Products were chilled with cold water for 10 min before storage for 24 h at 0ºC.

C. Product inoculation Individual batches of chilled uncured turkey rolls were removed from the bags, cut into small pieces and placed in a food processor (Tefal Kaleo Type 676041, Veenendal, the Netherlands). Approximately 6 ml (1% v/w final product) of an appropriate dilution of the five-strain mixture was added to the meat (600-g bulk) to reach 3 log CFU/g and homogenized into a coarse-chopped consistency. The chopped product was divided into 25-g samples and vacuum-packaged in filter bags (Stomacher bag with lateral filter, M-Tech Diagnostics, Ltd., Cheshire, UK) and stored at 7ºC for up to 97 d.

D. Physical properties Determination of cooking yields (%) of turkey rolls from different batches was based on product weight before and after cooking and chilling. The pH of the samples was measured by placing approximately 25 g of sample in a sterile filter bag (M-Tech) and homogenizing (Stomacher 400 Lab Blender, Seward Medical, London, England) with distilled water (1:10) and immersing a pH electrode (744 pH, Metrohm, Herisau Switzerland) in the bag containing the homogenate. The water activity of the turkey rolls was determined using an Aw Sprint TH 500 (Novasina, Talstrasse, Switzerland) by placing approximately 5-g portions of chopped meat into a plastic sample cup and inserted into the vapor chamber. Moisture/loss on drying (LOD) was determined within 24 h of production using an oven method (16h at 80ºC).

E. Microbiological analyses Duplicate samples of each treatment were analyzed on days 0, 3, 6, 10, 14, 20, 24, 31, 45, 63, and 97 on tryptic soy agar (TSA, Difco) for total bacterial populations and onto PALCAM agar (Difco) for L. monocytogenes populations. Each sample (25-g chopped turkey roll) was opened and sterile diluent (8.5% w/w sodium chloride and 0.1% w/v bacteriological peptone) added in a ratio of 1:3 (meat:diluent) and homogenized for 60 s (Seward Medical). Additional dilutions were also made in the same sterile diluent. A 50 µl portion of the appropriate dilution for each sample was plated (onto TSA and PALCAM) using a spiral plater (Eddyjet type 1.23, IUL Instruments, Barcelona, Spain). Plates were incubated for 48 h at 30ºC and the colonies counted using an automatic colony counter (Colyte Supercount, Synoptics, Cambridge, UK) and the associated software package.

F. Statistical analysis The experiment was conducted twice, and for each replicate two individual samples (25-g samples) were analyzed on each sampling day for each treatment (n=4). The microbiological data were converted to log CFU/g based on the sample weight analyzed and the volume of diluent added to each sample. Data for changes in populations of L. monocytogenes were analyzed by one-way analysis of variance to determine significant differences among antimicrobial treatments at each weekly sampling interval using Minitab version 14.2 statistical software (Minitab, Inc., State College, PA). Differences were considered significant when the associated P value was less than 0.05.

III. RESULTS AND DISCUSSION

The physical properties of the turkey rolls are presented in Table 1.

Table 1. Yield (%), moisture/loss of drying (LOD, %w/w), a_w, and pH of uncured turkey rolls formulated with 0.3% coated citric acid (CCA), 1.4% potassium lactate and 0.1% sodium diacetate (PLSD), or CCA+PLSD and determined 24 h after cooking

	Yield (%)	Moisture/LOD (%w/w)	a_w	pH
CCA	100±0	70.7±0.3	0.970±0.012	5.78±0.13
PLSD	100±0	69.4±0.4	0.966±0.010	6.22±0.02
CCA+PLSD	100±0	69.3±0.6	0.964±0.009	5.74±0.08

The pH of the brine containing CCA, PLSD, and CCA+PLSD was 7.77±0.12, 6.08±0.20, and 6.09±0.18, respectively (data not shown). As expected, the final product formulated with CCA and PLSD+CCA had a lower pH than that formulated with PLSD alone (by 0.44-0.48 units) (Table 1); indeed this was the purpose of including a "delayed-release" organic acid that would release after cooking and as such lower the final product pH to enhance the antilisterial effect of the lactate/diacetate blend without potential negative effects on cooking yield and texture. Previous trials in our lab (data not shown) indicated that use of CCA (as an acidulant) with PSLD did not negatively impact texture (consistent meat emulsion and typical structure) and yield (100%) while use of lactic and gluconic acid combined with PSLD had a significant negative effect on product texture (inconsistent meat emulsion and high purge final product) and yields of 76 and 86%, respectively. In this study, the aw, moisture, and yield of CCA, PLSD, and CCA+PLSD did not differ after cooking although the pH of the formulations containing CCA was lower indicating that the desired objective was achieved (Table 1). It should also be noted that although not part of the current study, previous work conducted by the researchers indicated no negative effects of the flavor acceptability of turkey rolls with the inclusion of CCA (in addition to PLSD) compared to turkey rolls with PLSD alone (data not shown). The antilisterial effect of the formulations is presented in Figure 1.

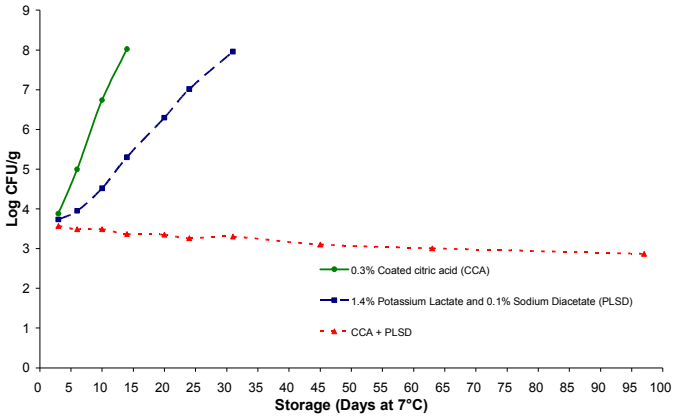

Figure 1. Mean populations of *Listeria monocytogenes* (PALCAM) inoculated (at approximately 3 log CFU/g) on chopped uncured turkey roll samples formulated with 0.3% coated citric acid (CCA), 1.4% potassium lactate and 0.1% sodium diacetate (PLSD), or CCA+PLSD, vacuum-packaged, and stored at 7ºC for up to 97 days.

Results represent counts obtained on PALCAM since there was no significant difference between these and the total bacterial populations. Use of only CCA in the product formulation resulted in growth of L. monocytogenes to 8 log CFU/g by day-14. Product formulation with PLSD alone experienced outgrowth of L. monocytogenes to 8 log CFU/g by day-31. The combination of PLSD and CCA provided complete inhibition of L. monocytogenes growth and even a bactericidal effect (reduction of 0.7 log CFU/g) through 97 d of storage at 7ºC. With the exception of day-0, the antilisterial effect of the formulations in the uncured turkey roll samples decreased ($P<0.05$) as follows: CCA+PLSD > PLSD > CCA Since there was little to no antilisterial activity of CCA alone, moderately more with PLSD alone, but complete inhibition of growth of L. monocytogenes through 97 d at 7ºC it appears that the inclusion of CCA with a lactate/diacetate blend has a primary function of regulating pH in the final product to optimize or enhance the antilisterial effect of the salt blend. Additionally, the L. monocytogenes present on the surface of the uncured turkey roll formulated with CCA+PLSD experienced a listericidal effect rather than just controlled outgrowth by the growth-inhibiting salts which suggests that the acid potentially injured a portion of the pathogen cells and the continued effect of the salt blend resulted in cell death of the injured cells. This however is speculation since the contribution of growth-inhibiting salts to death of injured cells was not studied. Future research into the possible role of growth-inhibiting salts on acid-injured bacterial (L. monocytogenes) cells is interesting and needed.

I. CONCLUSION

Combining a delayed-release organic acid, in the form of coated citric acid, with a lactate/diacetate blend enables meat processors to inhibit the outgrowth and even result in slight reductions of L. monocytogenes populations on uncured turkey rolls stored up to 97 d under vacuum at 7ºC. The use of a delayed-release (latent) organic acid enhances the antilisterial of organic acid salt(s) in the formulation of RTE meat enabling processors to control L. monocytogenes outgrowth on their products, especially uncured meats, without having to increase the level of the salt blend.

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