Effects of Lactic Acid on the Growth Characteristics of Listeria monocytogenes on Cooked Ham Surfaces†

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ABSTRACT

The surfaces of ready-to-eat meats are susceptible to postprocessing contamination by Listeria monocytogenes. This study examined and modeled the growth characteristics of L. monocytogenes on cooked ham treated with lactic acid solutions (LA). Cooked ham was inoculated with L. monocytogenes (ca. 10⁴ CFU/g), immersed in 0, 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0% LA for 30 min, vacuum packaged, and stored at 4, 8, 12, and 16°C. LA immersion resulted in <0.7 log CFU/g immediate reduction of L. monocytogenes on ham surfaces, indicating the immersion alone was not sufficient for reducing L. monocytogenes. During storage, no growth of L. monocytogenes occurred on ham treated with 1.5% LA at 4 and 8°C and with 2% LA at all storage temperatures. LA treatments extended the lag-phase duration (LPD) of L. monocytogenes and reduced the growth rate (GR) from 0.21 log CFU/day in untreated ham to 0.13 to 0.06 log CFU/day on ham treated with 0.5 to 1.25% LA at 4°C, whereas the GR was reduced from 0.57 log CFU/day to 0.40 to 0.12 log CFU/day at 8°C. A significant extension of the LPD and reduction of the GR of L. monocytogenes occurred on ham treated with >1.25% LA. The LPD and GR as a function of LA concentration and storage temperature can be satisfactorily described by a polynomial or expanded square-root model. Results from this study indicate that immersion treatments with >1.5% LA for 30 min may be used to control the growth of L. monocytogenes on cooked meat, and the models would be useful for selecting LA immersion treatments for meat products to achieve desired product safety.

Listeria monocytogenes is a foodborne pathogen capable of causing severe illnesses. This pathogen is frequently detected in fresh and prepared meats, raw or inadequately heat-treated milk and cheese products, seafoods, fruits, vegetables, and the processing environment (8). Researchers have reported L. monocytogenes prevalence rates of 4.2 to 8.0% in sliced luncheon meats (20), 0.89% in ready-to-eat (RTE) luncheon meats (13), 1.6% in packaged frankfurters (35), and 4.9% in cooked meat products (34). Contamination of RTE meat, beef, and poultry products with L. monocytogenes has resulted in class I food recalls of RTE meats in the United States (32). Refrigerated RTE meat products contaminated with L. monocytogenes were implicated in several outbreaks of severe listeriosis (9). A recent outbreak in Canada was linked to the consumption of contaminated deli meats and included 57 illnesses and 22 deaths (36). In a risk assessment, among 20 RTE food categories deli meats had the highest risk of causing illness and death from L. monocytogenes (33).

L. monocytogenes contamination of RTE meats is mostly limited to the surfaces of products and usually occurs during postcooking steps such as slicing and packaging. Several antimicrobials have been evaluated as additives for controlling L. monocytogenes in the formulation of RTE meats, and salts of lactate and diacetate are among the most effective and commonly used antimicrobials, especially when added in combination to RTE meats. Several studies have been conducted on lactate and diacetate blends in the product formulation (2, 12, 21, 22, 30, 38). Hot water, steam, electrolytic water, organic acids, and ozonated water spray have been used to decontaminate the surfaces of beef and pork carcasses and raw poultry products (1, 14, 17, 23). Among organic acids, lactic acid (LA) is most commonly used for treating animal carcasses, and a 2% LA wash is often used in meat processing plants for treating beef carcasses (2, 11, 19, 31). Dipping and spray washing with solutions of organic acids and their salts (4, 5, 24, 27) combined with other antimicrobials (12, 26) and addition of these compounds to product formulas (2) also have been evaluated on raw and processed meat products. In turkey roll that was inoculated with L. monocytogenes, spray washing with 2% LA at 55.4°C for 20 s significantly reduced the level of this pathogen on the meat surfaces, and LA was more effective than acetic acid or levulinic acid for reducing L. monocytogenes on the surfaces (5). However, L. monocytogenes was able to grow on the LA-washed turkey roll during subsequent storage at 4°C for 16 weeks. During spray washing, the acids are generally applied to meat...
surfaces for only a short period of time, e.g., 15 to 40 s (5, 7, 18). For meat products with complex surface morphology and large surface areas, immersion sanitation is easier to perform and a spray apparatus is not needed. Immersion also allows for a longer treatment time and requires less of the antimicrobial for treatment. Dipping in organic acid has been used for controlling L. monocytogenes on processed meats; in general, the acid treatments were applied for a short period of time, e.g., <5 min (2, 26, 37).

The objectives of the present study were to examine the effect of a lengthy (30-min) immersion treatment in LA on the growth characteristics of L. monocytogenes on ham surfaces stored at refrigeration and abuse temperatures and to develop predictive models of these growth characteristics. These models could be useful in the field of predictive microbiology.

**MATERIALS AND METHODS**

*L. monocytogenes inoculum*. Five strains of *L. monocytogenes* obtained from the culture collection of the Residue Chemistry and Predictive Microbiology Research Unit (U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA) were used in this study: H7776 (4b, frankfurter isolate), MFS 2 (1/2a, pork processing plant environment isolate), Scott A (4b, clinical isolate), 101M (4b, beef and pork sausage isolate), and F2365 (Hispanic-style cheese isolate). Each strain was transferred from a ~80°C stock culture into 10 ml of brain heart infusion (BHI) broth (BD, Franklin Lakes, NJ) and incubated at 37°C overnight. A loopful of the overnight culture was transferred to another 10 ml of BHI broth and incubated at 37°C for 24 h. One-milliliter aliquots of the cell suspension from each strain were mixed together, and this mixture was diluted with sterile 0.1% (wt/vol) peptone water (PW) to obtain an *L. monocytogenes* inoculum of approximately 10^6 CFU/ml.

**LA solutions.** A 0.8-LA solution (85%; Sigma Chemicals, St. Louis, MO) was diluted with sterile distilled water to prepare 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0% LA solutions. The pH values for the 0.5, 1.0, 1.5, and 2.0% LA solutions were 3.1, 2.8, 2.4, and 2.3, respectively.

**Sample preparation.** A cooked ham product (76% moisture, 2.9% fat, 16.5% protein, and 1.9% salt) purchased from a local supplier was used as a food model in this study. The ham product contained no added preservatives such as lactate or diacetate, as indicated by its ingredient label. To ensure that the survival or growth of *L. monocytogenes* on the ham surfaces was affected only by the LA treatment and not by other factors (e.g., outgrowth of the native microflora), ham pieces (1 by 1 by 1 cm) were sealed in stomacher bags, heated in a water bath to 63°C, and held for 30 min to inactivate the native microflora. After heat treatment, ham pieces were cooled under running water. The weight of a piece of ham was approximately 0.7 g. A 100-g sample of ham was placed into a sterile polypropylene container (Fisher Scientific, Pittsburgh, PA), and 100 ml of *L. monocytogenes* inoculum was added to completely cover the ham piece. After 30 min, the inoculum was drained, and 100 ml of 0.5, 0.75, 1.0, 1.25, 1.5, or 2.0% LA was poured into the container. After 30 min, the LA solution was drained from the container and the ham pieces were left at room temperature for 30 min. The ham pieces retained approximately 0.8% of the LA solution. For pH measurements, approximately 3 g of ham and an equal amount of distilled water were placed into a 100-ml stomacher bag and mixed in a BagMixer 400 stomacher (Interscience, St. Nom, France) for 1 min. The mixing did not break up the meat pieces but released surface liquid, and the pH was determined with a SevenMulti pH meter fitted with an InLab RoutinPro pH electrode (Mettler-Toledo, Schwerzenbach, Switzerland). The pH values of the liquid residue on ham surface treated with 0.5, 1.0, 1.5, and 2.0% LA solutions were 4.6, 4.4, 3.9, and 3.6. Ham pieces (3 to 4 g) were placed into 100-ml stomacher bags (Spiral Biotech Inc., Norwood, MA) and vacuum sealed with an A300 vacuum sealer (Multivac Inc., Kansas, MO) to ~980 mbar. These sealed samples were stored at 4, 8, 12, and 16°C for up to 42 days. The experiment was performed in two trials with two samples prepared for each sampling interval during each trial.

**Enumeration of *L. monocytogenes*.** Populations of *L. monocytogenes* on ham during storage were enumerated periodically. Samples from two bags were added to an equal weight of sterile 0.1% PW and stomached for 2 min. Additional dilutions were prepared in sterile 0.1% PW, and duplicate 50-μl dilutions were spread plated on PALCAM agar containing PALCAM antimicrobial supplement (BD). Plates were incubated at 37°C for 48 h, and gray colonies surrounded by a black precipitate were counted. Periodically, sample dilutions were also spread plated on tryptic soy agar (BD) to determine whether samples were contaminated. When microbial contamination was observed, samples for the treatment were discarded, and a new batch of samples was prepared.

**Estimation of LPD and growth rate of *L. monocytogenes*.** The log-transformed populations of *L. monocytogenes* on ham were plotted against storage time (days), and the growth curves were fitted with the three-phase linear model of Buchanan et al. (3) to estimate the lag-phase duration (LPD in days) and growth rate (GR in log CFU per day):

\[
\text{Lag phase: } \text{For } t < t_{lag}, \quad N_t = N_0
\]

Exponential growth phase:

\[
\text{For } t_{lag} < t < t_{max}, \quad \text{GR} = (N_t - N_0) / (t - t_{lag})
\]

where \(N_0\) is the initial population (log CFU per gram), \(N_t\) is the population at sampling time \(t\), and \(t_{lag}\) is the LPD (days). The GR was estimated from the slope of the regression line of the exponential growth phase, and the LPD was the storage time on the growth curve where the slope intercepts the initial population.

**LPD and GR as a function of LA concentration and storage temperature.** The LPD and GR of *L. monocytogenes* on ham as a function of LA concentration and storage temperature were analyzed using the general linear model and nonlinear regression procedures of SAS 9.1 for Windows (SAS Institute Inc., Cary, NC). The general linear model was used to fit the LPD or GR with the following quadratic equation:

\[
\text{LPD or } \text{GR} = \alpha + \beta_1 \times \text{LA} + \beta_2 \times \text{temperature} + \beta_3 \times \text{LA} \times \text{temperature} + \beta_4 \times (\text{LA}^2) + \beta_5 \\
\times \left(\text{temperature}\right)^2
\]

where \(\alpha\) is the intercept and \(\beta_1\) through \(\beta_5\) are estimated coefficients.

The nonlinear regression procedures with the Gauss-Newton iterative method was used to fit the GR with an expanded square-root model (10):

\[
\sqrt{\text{GR}} = a \times (T - T_{min})^b \times (\text{LA}_{max} - \text{LA})^c
\]

where \(a, b, \text{ and } c\) are fitting coefficients, \(T\) is the storage temperature, \(\text{LA}\) is the LA concentration, \(T_{min}\) is the nominal...
RESULTS AND DISCUSSION

Effect of LA immersion on initial reductions of L. monocytogenes. The initial L. monocytogenes inoculum level on ham before LA treatment was approximately 3.2 log CFU/g. L. monocytogenes reduction was 0.1 to 0.6 log CFU/g after LA treatment, and greater reductions were obtained with higher LA concentrations. The L. monocytogenes reductions on ham after 30 min of immersion in 2% LA were comparable to the 0.6-log reduction of L. monocytogenes reported for turkey roll after a spray wash with 2% LA for 20 s at 20 lb/in² pressure and 55.4°C (5). The longer LA immersion time had an effect on the initial reduction of L. monocytogenes on meat surfaces similar to that of a short spray wash. The reductions of L. monocytogenes, Escherichia coli O157:H7, and Salmonella on raw or processed meats and poultry products after treatment with 1 to 3% organic acid spray were generally less than 2.0 log units (5, 11, 29). Longer immersion time did not appear to improve the efficacy of surface decontamination over that of acid spray wash. The reduction of pathogens by spray wash was due to the physical removal of bacterial cells from the meat surfaces by the acid stream and to the bactericidal effects of low pH and high temperature (mostly <55°C) on the meat surfaces (1, 6, 7). Although the immersion in the present study was static without the flow of the acid solution along the meat surface, the longer contact time (30 min) and the low pH of the LA solutions most likely contributed to the reduction of L. monocytogenes to a level similar to that achieved with the LA spray wash for 20 s at 55°C (5). Because of the limited reduction of L. monocytogenes achieved with the LA immersion treatment, this approach was not considered an effective means of reducing L. monocytogenes immediately after inoculation. Byelashov et al. (4) reported a model that can be used to predict L. monocytogenes reductions on a frankfurter surface as a function of the concentration of the LA solution (0 to 3%), solution temperature (4, 25, 40, and 55°C), and immersion time (0 to 2 min). The model indicates that higher LA concentrations and solution temperatures and longer immersion times result in greater reductions of L. monocytogenes.

Survival and growth of L. monocytogenes on ham. The effects of LA immersion treatment on the subsequent growth or survival of L. monocytogenes on ham surfaces at 4, 8, 12, and 16°C are shown in Figure 1. L. monocytogenes was not able to grow on ham treated with 1.5% LA and stored at 4 and 8°C and on ham treated with 2% LA and stored at all temperatures. The growth of L. monocytogenes was slower on ham treated with higher LA concentrations and stored at lower temperatures. On ham without LA treatment, the population of L. monocytogenes reached approximately 6.0 log CFU/g after 24, 6, 5, and 4 days at 4, 8, 12, and 16°C, respectively. The storage time needed for L. monocytogenes to reach a similar population was extended on ham treated with LA, and the time increased as the LA concentration increased. The results indicated that LA immersion treatment reduced the growth of L. monocytogenes at refrigeration and abuse temperatures. Immersion in 1.5% LA for 30 min inhibited the growth of L. monocytogenes at 4 and 8°C, whereas 2% LA inhibited growth at higher abuse temperatures. Samelis et al. (28) examined the survival of L. monocytogenes, E. coli O157:H7, and Salmonella Typhimurium DT104 in rinsates collected from fresh beef rounds spray washed with 2% LA. The three pathogens were not able to grow and did not survive for more than 2 days in rinsates stored at 4 or 10°C. The efficacy of the 30-min immersion in 2% LA for controlling the growth of L. monocytogenes on ham surfaces may due to the longer immersion time; surface treatments with LA concentrations of 2% or higher for shorter times have been ineffective for inhibiting the growth of L. monocytogenes on meat surfaces. In one study, L. monocytogenes on the surfaces of turkey roll treated with 2% LA spray wash for 20 s increased from 4.2 to 6.8 log CFU/g after 8 weeks at 4°C (5). Samelis et al. (27) reported that the population of L. monocytogenes on the surfaces of vacuum-packaged bologna slices treated with 2.5% LA by dipping for 1 min increased from approximately 3.0 to 6.0 log CFU/cm² after 35 days at 4°C. Barmpalia et al. (2) found that L. monocytogenes was able to grow on frankfurters dipped for 2 min in 2.5% LA or acetic acid when the product was stored at 10°C. In a study of the growth probability of L. monocytogenes on ham and turkey breast products as a function of LA concentration (0 to 4%), dipping time (0 to 4 min), and storage temperature (4 to 10°C), Yoon et al. (37) developed a mathematical model to describe the growth boundary of L. monocytogenes in both products and concluded that higher LA concentrations and/or longer dipping times reduced the growth probability of L. monocytogenes. A longer acid contact time probably increases the acidification of the meat surfaces and subsequent injury of bacterial cells, reducing their ability to survive or grow on the meat surfaces.

The LA immersion treatment generally imparted a slightly acidic taste to the meat pieces and reduced the pinkish color of the meat surfaces during storage. A sensory evaluation indicated that the treatment could be suitable for selected applications such as cooked meats used in salads or meat products, such as sliced ham, that contain only a small portion of the treated surface.

Effect of LA on the LPD and GR of L. monocytogenes. The estimated LPD and GR of L. monocytogenes on ham treated with 0 to 1.5% LA and stored at 4 to 16°C are shown in Table 1. The LPD of L. monocytogenes on ham
treated with 0.5 to 1.5% LA were 1.8 to 42 days at 4°C, 1.4 to 42 days at 8°C, 1.1 to 32 days at 12°C, and 0 to 24 days at 16°C. Figure 2A shows the plot of response surface of the LPD. Treatments with higher LA concentrations increased the LPD of *L. monocytogenes*. The average increases in LPD at increasing LA concentrations are shown in Table 2. The increase in LPD was more profound at 1.25 to 1.5% LA at all storage temperatures. The GR of *L. monocytogenes* on ham treated with 0.5 to 1.25% were 0.13 to 0.06 log CFU/day at 4°C, 0.40 to 0.12 log CFU/day at 8°C, 0.74 to 0.22 log CFU/day at 12°C, and 0.98 to 0.33 log CFU/day at 16°C. The decrease in GR was linear in relation to the decrease in storage temperature and nonlinear in relation to the increase in LA concentration (Fig. 2B). The largest average decreases of GR were -0.02 log CFU/day for 1.25 to 1.5% LA and storage at 4°C, -0.05 log CFU/day for 1.0 to 1.25% LA and storage at 8°C, -0.08 log CFU/day for 0.75 to 1.0% LA and storage at 12°C, and -0.10 log CFU/day for 1.0 to 1.25% LA and storage at 16°C (Table 2). The decrease of GR was more pronounced at >1.25% LA, and at higher storage temperatures the average reduction of GR was more significant at higher LA concentrations. A greater increase in LPD and reduction of GR of *L. monocytogenes* occurred on ham treated with LA at concentrations higher than 1.25%.

The LPD and GR of *L. monocytogenes* on ham surfaces treated with LA and stored at 4 to 16°C were modeled to develop mathematical equations for applications in predictive microbiology. The LPD in original and natural logarithm (base e) forms were fitted with equations 1 and 2. The resulted equations were not satisfactory as judged by the residues (differences between predicted and observed values). A fitting of the polynomial equation with logarithm (base 10)–transformed LPD was more suitable:

$$\log(\text{LPD}) = 0.177 - 9.937 \times \text{LA} - 0.0147 \times \text{temperature} - 2.5457 \times \text{LA} \times \text{temperature} + 8925.626 \times (\text{LA})^2 + 0.00012 \times (\text{temperature})^2$$

Both LA concentration and storage temperature significantly affected (*P* < 0.05) the LPD of *L. monocytogenes*. The plot of observed versus predicted values and the regression line are shown in Figure 3A. The regression line has a regression coefficient ($R^2$) of 0.98 and a slope of 1.01, indicating that the predictive values are close to the observed values. The bf is 1.0045 and the af is 1.18, indicating that the model overestimates the LPD by an average of 18%. The overestimation of LPD is a fail-dangerous situation. Therefore, when using the model to estimate the LPD of *L. monocytogenes* on ham surfaces, the overestimation must be taken into consideration and the estimated LPD adjusted accordingly. A quadratic model also has been used to describe the LPD of *L. monocytogenes* in modified-atmosphere-packed...
cooked meat as a function of water activity, temperature, dissolved carbon dioxide, and sodium lactate. The LPD were natural logarithm transformed before fitting, and the model was better for describing the LPD than was a modified square-root model (10).

The GR values were transformed to square-root values and fitted with polynomial (equation 1) and expanded square-root (equation 2) models. Fitting the square-root model with exponents for temperature and LA at 1 and 0.5, respectively, produces a nominal minimal growth temperature and a maximum growth LA concentration of −3.9°C and 1.56%, respectively (equation 4):

$$\sqrt{GR} = 0.0496 \times [T - (-3.9)] \times (0.0156 - LA)^{0.5} \quad (4)$$

The minimal growth temperature and maximum growth LA concentration are close to the reported −0.4°C (16) and 1.5% LA that inhibited the growth of *L. monocytogenes* on ham at 4 and 8°C in the present study. The model has a bf of 0.97 and an af of 0.97, indicating the model underestimates the GR by an average of 3%. The plot of observed versus predicted values is linear, with a regression coefficient of 0.97 and a slope of 0.97 (Fig. 3B). Although the model is acceptable, the $T_{min}$ is below the freezing point. A new model derived from the square-root model (temperature alone), on which the expanded square-root models are based, revealed that the 1.5 exponent for the temperature parameter produced minimal growth temperatures for bacteria that were closer to those reported in the literature (15). With a modification to the expanded square-root model, GR was refitted with the exponents for temperature and LA selected by the regression procedure. The resulted parameter estimates are shown in equation 5:

$$\sqrt{GR} = 1.1929 \times (T - 1.3)^{0.5655} \times (0.0151 - LA)^{0.3707} \quad (5)$$

The $T_{min}$ is 1.3°C and $L_{max}$ is 1.51%, and the model has a bf of 1.09 and an af of 0.96. The model overestimates the GR by an average of 4%. The regression coefficient is 0.97, and the slope is 1.01 for the regression line (Fig. 3B). The GR values were also fitted to equation 1:

$$GR = 0.1472 + 22.182 \times LA + 0.0782 \times temperature - 2.158 \times LA \times temperature - 2.943.523 \times (LA)^2 - 0.0009 \times (temperature)^2 \quad (6)$$

The bf of this model is 1.07 and the af is 0.98, indicating the predicted values from the model are an average of 2% higher than the observed values. The regression of predicted versus observed values is linear ($R^2 = 0.98$) with a slope of 0.99. Both of the quadratic and expanded square-root models satisfactorily describe the GR of *L. monocytogenes* on ham at 4 to 16°C as affected by the LA treatments. Devlieghere et al. (10) also reported that the maximum GRs of *L. monocytogenes* in modified-atmosphere-packaged cooked meat were well described by an
expanded square-root model and a quadratic model. Although both model types are equally suitable for describing the GR of *L. monocytogenes* on meat products, the simpler form of the expanded square-root model is preferred. In addition, the parameters of $T_{\text{min}}$ and $L_{A_{\text{max}}}$ provide additional biological meaning to the model.

The results of this study revealed that immersion in LA at concentrations higher than 1.5% may be used to enhance the microbiological safety of cooked ham products that are susceptible to surface contamination by *L. monocytogenes*. The LPD and GR models could be used to select LA immersion treatments for cooked ham products to achieve the desired product safety.

**REFERENCES**


