Effect of Combining Nisin and/or Lysozyme with In-Package Pasteurization on Thermal Inactivation of Listeria monocytogenes in Ready-to-Eat Turkey Bologna†

SUNIL MANGALASSARY,1 INYEE HAN,1 JAMES RIECK,2 JAMES ACTON,1 XIUPI NG JIANG,1 BRIAN SHELDON,3 AND PAUL DAWSON1*

1Department of Food Science and Human Nutrition and 2Department of Applied Economics and Statistics, Clemson University, Clemson, South Carolina 29634-0371; and 3Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695, USA

MS 07-117: Received 2 March 2007/Accepted 3 June 2007

ABSTRACT

Achieving a targeted lethality with minimum exposure to heat and preservation of product quality during pasteurization is a challenge. The objective of this study was to evaluate the effect of nisin and/or lysozyme in combination with in-package pasteurization of a ready-to-eat low-fat turkey bologna on the inactivation of Listeria monocytogenes. Sterile bologna samples were initially treated with solutions of nisin (2 mg/ml = 5,000 AU/ml = 31.25 AU/cm²), lysozyme (10 mg/ml = 80 AU/ml = 0.5 AU/cm²), and a mixture of nisin and lysozyme (2 mg/ml nisin + 10 mg/ml lysozyme = 31.75 AU/cm²). Bologna surfaces were uniformly inoculated with a Listeria suspension resulting in a population of approximately 0.5 log CFU/cm². Samples were vacuum packaged and subjected to heat treatment (60, 62.5, or 65°C). Two nonlinear models (Weibull and log logistic) were used to analyze the data. From the model parameters, the time needed to achieve a 4-log reduction was calculated. The nisin-lysozyme combination and nisin treatments were effective in reducing the time required for 4-log reductions at 62.5 and 65°C but not at 60°C. At 62.5°C, nisin-lysozyme–treated samples required 23% less time than did the control sample to achieve a 4-log reduction and 31% less time at 65°C. Lysozyme alone did not enhance antilisterial activity with heat. Results from this study can be useful to the industry for developing an efficient intervention strategy against contamination of ready-to-eat meat products by L. monocytogenes.

Postcooking contamination of ready-to-eat (RTE) meat and poultry products by Listeria monocytogenes is a major food safety problem and an economic hardship for the food industry. L. monocytogenes is an important foodborne pathogen that can cause life-threatening invasive infections in neonates, pregnant women, elderly, and immunocompromised individuals (6, 33). The majority of the food product recalls associated with listeriosis involve RTE meat and poultry products. These food product recalls have serious economic and public perception impacts on the food industry (9, 11, 14, 35, 36).

Postprocess operations such as peeling, sorting, loading, and slicing during preparation of RTE meat products are potential sources for recontamination of products with L. monocytogenes (22). The organism can survive in food-processing facilities for long periods (37), and thus processing equipment and other food contact surfaces can act as a source for recontamination during postprocess operations.

In 2003, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service released an interim final rule for control of L. monocytogenes with the use of postlethality treatments in RTE meat and poultry products (39). This ruling includes three alternative approaches that establishments can implement during processing of RTE meat and poultry products involving a postlethality treatment, antimicrobial agent, and/or sanitation control measures.

Heat treatment in the form of pre- and postpackage pasteurization with hot water or steam is an effective method for reducing postprocess surface contamination by L. monocytogenes (16, 19, 20, 30). The effects of various factors, such as product thickness, product composition, product surface characteristics, and packaging film thickness, on the efficiency of in-package pasteurization also have been investigated (13, 21). Another important factor is the time or duration of pasteurization; processors prefer shorter treatments because they are more cost-effective and less detrimental to product quality.

Surface application of antimicrobial agents singly or in combinations also is effective for controlling L. monocytogenes in RTE meats (7, 31). Nisin and lysozyme are two antimicrobial agents approved for many food applications. Nisin has synergistic antibacterial activity with heat against L. monocytogenes (2, 10, 18). This synergism may yield targeted lethality in a shorter time when nisin is combined with in-package surface pasteurization. Enhanced antibacterial activity of a mixture of nisin and lysozyme against pathogenic and spoilage bacteria has been reported (4, 7, 24). However, studies to determine the synergy of this mixture in combination with heat have not been conducted.
Considering the reported synergy of various intervention methods, in-package pasteurization combined with pre-surface application of nisin and lysozyme may have an increased effect for achieving a targeted bacterial reduction with shorter pasteurization treatment. The objective of this study was to evaluate the surface application of nisin and/or lysozyme in combination with in-package pasteurization of RTE low-fat turkey bologna to eliminate \( L. \) monocytogenes, particularly noting changes in the heat inactivation kinetics of the organism.

**MATERIALS AND METHODS**

**Food product preparation.** RTE low-fat turkey bologna (average 14.3% fat, 10.7% protein, and 71.4% moisture, with 2% salt) was used for the experiment. Bologna samples were batch irradiated for 521 min with a cobalt 60 source for a total dose of 2.4 kGy at 4,607 R/min at Auburn University (Auburn, Ala.) then shipped frozen to Clemson University (Clemson, S.C.). Irradiation was carried out to eliminate background microflora before inoculation studies were conducted. Bologna samples were kept frozen at \(-70^\circ\text{C}\) and then thawed overnight at \( 4^\circ\text{C} \) before experimentation. For inoculation and thermal inactivation studies, bologna slices approximately 2.5 mm thick were cut into pieces measuring 4 by 4 cm with a sterile cutting template, and each piece was considered an experimental unit.

**Culture preparation.** \( L. \) monocytogenes ATCC 15313 was preserved by freezing the cultures at \(-70^\circ\text{C}\) in vials containing brain heart infusion (BHI) broth (Becton Dickinson, Sparks, Md.) supplemented with 20% (vol/vol) glycerol (Sigma, St. Louis, Mo.). To propagate the culture, a frozen vial was thawed at room temperature, and 0.1 ml of the thawed culture was transferred to 9.9 ml of BHI broth in screw-cap tubes and incubated aerobically for 16 to 18 h at \( 37^\circ\text{C} \) with agitation at 200 revolutions per min (Thermolyne Maxi-Mix III type 65800, Barnstead/Thermolyne, Dubuque, Iowa). The inoculum was prepared from a second transfer of this culture (0.1 ml) to another 9.9 ml of BHI broth, which was incubated aerobically for 16 to 18 h at \( 37^\circ\text{C} \) with agitation. After incubation for 16 h, a washed cell suspension was harvested by centrifugation at 3,000 \( \times g \) (IEC HN-II centrifuge, International Equipment Co., Inc., Needham Heights, Mass.) and washed with 10 ml of 0.1% sterile Peptone water (Bacto Peptone, Becton Dickinson), and the pellet was resuspended in 0.1% sterile peptone water to obtain a population of approximately 8 to 9 log CFU/ml. Initial cell populations were verified by enumeration of the cells after pour plating on BHI agar and incubating at 37°C for 48 h.

**Antimicrobial activity assay.** Activities of nisin and lysozyme were determined by critical dilution assay (27). Serial two-fold dilutions of the antimicrobial agents were tested against \( L. \) monocytogenes ATCC 15313. Ten microliters of each dilution was spotted on the surface of the BHI agar medium seeded uniformly with a suspension of \( L. \) monocytogenes. After incubation (48 h at \( 37^\circ\text{C} \)), the plates were checked for zones of inhibition. Titors of nisin and lysozyme in arbitrary units (AU) per milliliter were expressed as the reciprocal of the highest dilution showing the zone of inhibition. Activity of the antimicrobial compounds was expressed in AU per milligram based on the weight of the antimicrobial compounds used in serial dilution and then converted into AU per milliliter based on the weight (milligrams) of the antimicrobial compound used in the application solution.

**Antimicrobial preparation.** Nisaplin, a commercial nisin product (2.5% nisin, 10\(^{6}\) IU/g), was provided by Danisco (New Century, Kans.). Solutions of required concentrations of nisin were prepared on the day of the experiment by dissolving the appropriate amount of nisin in sterile distilled water. Egg white lysozyme was provided by Q.P. Corporation (Tokyo, Japan). Solutions of lysozyme were prepared on the day of the experiment by dissolving appropriate amounts in sterile distilled water.

**Antimicrobial treatment and inoculation.** Sterile frozen bologna samples were thawed overnight at \( 4 \pm 1^\circ\text{C} \). On day 1 of the experiment, bologna samples were removed from the refrigerator and aseptically transferred to a sterile surface under a Bio-flow chamber (Germfree, Ormond Beach, Fla.). Each bologna slice was cut into pieces (4 by 4 cm) with a sterile cutting template as previously described. Meat samples for the thermal treatment were uniformly surface treated with 0.1 ml of the antimicrobial solutions. The treatments were control, 0 \( \text{AU/cm}^2 \); nisin, 31.25 \( \text{AU/cm}^2 \) (5,000 \( \text{AU/ml} = 2 \text{mg/ml} \)); lysozyme, 0.5 \( \text{AU/cm}^2 \) (80 \( \text{AU/ml} = 10 \text{mg/ml} \)); and nisin-lysozyme, 31.75 \( \text{AU/cm}^2 \) (5,000 \( \text{AU/ml} \) nisin + 80 \( \text{AU/ml} \) lysozyme). The levels of nisin and lysozyme were selected based on the initial experiments (data not shown), which were conducted to determine the optimal antimicrobial concentrations required to leave enough surviving cells to allow calculation of thermal inactivation models when combined with in-package pasteurization. After applying the antimicrobial treatment, all bologna surfaces were inoculated uniformly (spread with a sterile glass hockey stick) with 0.1 ml of a 9 log CFU/ml suspension of \( L. \) monocytogenes to yield a cell concentration of 0.5 log CFU/cm\(^2\). All samples were aseptically transferred to a postpasteurization bag (model CNP-310, Cryovac, Duncan, S.C.). Insulated thermocouples (K type Teflon, Omega Engineering, Stamford, Conn.) were attached to the surface of two uninoculated bologna samples to measure the temperature during thermal treatment. All bags were then vacuum packaged (model UV 250, Koch Supplies, Kansas City, Mo.).

**Thermal processing and heat resistance determination.** Packaged bologna samples were simultaneously submerged into a water bath (model 186, Precision Scientific Incorporated, Chicago, Ill.) and equilibrated to one of three predetermined temperatures (60, 62.5, or 65°C). The surface temperature and time data were monitored during the entire process using a channel datalogger (CALPlex 32, TechniCAL, New Orleans, La.) and thermal processing software (CALSoft version 1.32, TechniCAL). Once the temperature of the bologna surface reached the equilibrium temperature, samples from each treatment were removed from the water bath at selected time intervals (seven total samples in each run at 10-, 20-, and 120-s intervals for 65, 62.5, and 60°C, respectively) and rapidly cooled by immersion in an ice-water slurry for 10 s to minimize any further thermal inactivation. The time intervals at each temperature were selected so that there were at least three to five survival points on the inactivation curve. Each sample was subsequently aseptically removed from the pasteurization bag and homogenized in 99 ml of sterile 0.1% peptone water (Bacto Peptone, Becton Dickinson), and the homogenate was serially diluted in 0.1% peptone water, and appropriate serial dilutions were then pour plated in duplicate using BHI agar. Plates were incubated at 37°C for 48 h before colonies were enumerated. The limit of sensitivity for bacterial numbers was about 10\(^{2}\) CFU/ml. CFU counts were converted to log CFU per square centimeter of the sample before analysis of the data. One set of all four treatments was evaluated microbiologically without any heat treatment to determine the number of surviving cells at time 0.
Statistical analysis. The experiment was replicated three times at each pasteurization temperature. The data were analyzed separately to determine the initial inhibitory effect of antimicrobial agents without heat and the inhibitory effect of the antimicrobial-heat combinations. These data were incorporated into several thermal inactivation models and evaluated for which models best fit the resulting pattern.

A 2 × 2 factorial design was used to analyze the initial inhibitory effect of antimicrobial agents at 0 h before in-package pasteurization. Two levels of nisin and lysozyme (present, not present) and the nisin × lysozyme interaction were used in the model. Data were analyzed with an analysis of variance using the generalized linear model procedure of SAS (32). The least square difference (LSD) multiple comparison procedure was used to evaluate the significance ($P < 0.05$) of differences among means.

Bacterial lethality for the antimicrobial-heat treatment combination was determined based on the log reductions obtained at a particular cutoff time. Log reductions were calculated by subtracting the log count for each treatment after a specific heating time from the initial log count obtained for the untreated control sample before heating. The cutoff point for heating time for each temperature was selected as the time at which there were detectable cells for all four treatments, not the complete time of pasteurization (antimicrobial treatments resulted in reduction of L. monocytogenes cells to below detection levels at 60 and 65°C by the completion of pasteurization for 720 and 60 s, respectively). Treatment differences ($P < 0.05$) within each pasteurization temperature were evaluated using the LSD multiple comparison procedure.

Survivor log counts obtained for all treatments over time were tested for linearity using orthogonal polynomials. For nonlinear curves, various nonlinear mathematical models were tested to obtain a best fit for the data. A single model was not satisfactory to fit all survivor curves at the three different temperatures. Therefore, both a Weibull model and a modified log-logistic model were selected to model the data for all treatments and temperatures. The goodness of fit by the two models was determined using an extra sum of squares test (29). The model with the better fit for each treatment at a particular temperature was selected and used from that point forward.

The cumulative form of the Weibull distribution suggested by Peleg and Cole (26) was used to describe the survivor curves obtained at 62.5 and 65°C:

$$\log \frac{N}{N_0} = -bt^n$$

where $N_0$ is the initial number of cells after come-up time (which is the time required for the sample to reach the set temperature), $N$ is the number of surviving cells after an exposure time $t$, $b$ is the scale factor (which is a characteristic of time), and $n$ is the shape factor. A small value of time (0.1) was used to approximate $t = 0$.

A modified version of the original log-logistic model (5) with the following equation was used to fit the survivor curves obtained at 60°C:

$$\log \frac{N}{N_0} = \frac{A}{1 + e^{-\sigma \log(time) + 1}}$$

where $A$ is the difference between the lower and upper asymptotes, $\sigma$ is the maximum rate of inactivation (maximum slope of inactivation curve), and $\tau$ is the log time to the maximum rate of inactivation.

The PROC NLIN procedure of SAS (32) was used to fit both equations and to estimate the parameters for each treatment at a particular temperature. By using the estimated parameters in the equation of a specific model for a particular temperature-treatment combination, the time required for a specific log reduction was estimated by substituting the value for the targeted log reduction in place of the log reduction ratio ($\log N/N_0$). For the Weibull model, the time was estimated using the equation

$$t = \left(\frac{\log N/N_0}{b}\right)^{1/n}$$

where $N_0$ is the initial number of cells after come-up time, $N$ is the number of surviving cells after an exposure time $t$, and $b$ and $n$ are parameters from the Weibull model where $b$ is the scale factor (which is a characteristic of time) and $n$ is the shape factor (when $n = 1$, the curve is linear).

For the log-logistic model, the time was estimated by the following formula:

$$t = \left[\frac{10^{\log A/\log N_0} - 1}{(\log 1/\log A)^{1/\sigma} + 1}\right] - 1$$

where $A$ is the difference between lower and upper asymptotes, $\sigma$ is the maximum rate of inactivation, and $\tau$ is the log time to the maximum rate of inactivation. The variance for the estimated time was calculated using the delta method (1). Comparisons of the antimicrobial treatments at a particular temperature were made using Wald’s hypothesis test (29).

RESULTS AND DISCUSSION

Antimicrobial activity assay. Nisin activity as determined by critical dilution assay was $2.5 \times 10^3$ AU/ml. Because 2 mg/ml nisin was used, the activity of nisin in the solution was 5,000 AU/ml. Lysozyme activity was 8 AU/ml, and thus in the solution (10 mg/ml) used for the experiment, the activity was 80 AU/ml.

Temperature profile. The surface temperature profile of bologna samples was monitored throughout pasteurization for all three temperatures. Come-up time, which is the time required for the bologna surface to reach the set water bath temperature, did not differ among the three temperatures. The consistency in come-up times among samples was facilitated by the use of a single slice of bologna in each of the thermal studies. The average come-up time was 60 s, and surface temperature profiles did not fluctuate during the holding phase of the pasteurization process.

Initial inhibitory effect of antimicrobial treatments. Nisin and nisin-lysozyme treatments resulted in an $\sim 0.5$ to 0.6 log CFU/cm² reduction ($P < 0.05$) in cell counts almost immediately (at 0 h) compared with the control and lysozyme treatments (Table 1). The interaction effect between nisin and lysozyme was not significant ($P > 0.05$), indicating an additive antibacterial effect for the nisin-lysozyme combination at 0 h. In previous studies, a synergistic effect of nisin and lysozyme on spoilage and on pathogenic bacteria has been reported (4, 7, 24). The short exposure time used in the current study may have contributed to the small difference in population reduction of L. monocytogenes due to the antimicrobial treatments. Extended exposure times during refrigerated storage will be examined in a subsequent study.

Bacterial lethality for the antimicrobial-heat treatment combination. Log reductions attained by the com-
TABLE 1. Listeria monocytogenes populations on inoculated bologna slices in different treatment groups at 0 h before in-package pasteurization

| Treatment | Mean (±SD) bacterial count (log CFU/cm²)
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.38 ± 0.06 A</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>7.31 ± 0.16 AB</td>
</tr>
<tr>
<td>Nisin</td>
<td>6.99 ± 0.15 B</td>
</tr>
<tr>
<td>Nisin-lysozyme</td>
<td>6.71 ± 0.21 BC</td>
</tr>
</tbody>
</table>

a Control, no antimicrobial added; lysozyme, 0.5 AU/cm² (80 AU/ml); nisin, 31.25 AU/cm² (5,000 AU/ml); nisin-lysozyme, 31.75 AU/cm² (5,080 AU/ml).

b Means with different letters are significantly different (P < 0.05).

The enhanced inhibitory effect of in-package pasteurization combined with various antimicrobial agents was investigated in previous studies (3, 17, 23). Chen et al. (3) combined the surface application of pediocin (ALTA 2341) with postpackaging thermal pasteurization in hot water at 71, 81, or 96°C for 30, 60, or 120 s to control L. monocytogenes on frankfurters. L. monocytogenes populations were reduced by all treatments, but 81°C or higher temperatures for at least 60 s in combination with pediocin were necessary to achieve at least a 50% reduction in the initial populations. In the present study, nisin and nisin-lysozyme treatments resulted in reductions of L. monocytogenes cells to below detectable levels (less than 0.0625 log CFU/cm²) on the bologna surface after pasteurization for 60 s at 65°C. Food product type, packaging materials, and antimicrobial agents influence the thermal inactivation of the organism during in-package pasteurization and are likely the reason for differences in the degree of inactivation detected across similar applications (13, 21).

Modeling of inactivation curves. Statistical analysis of the survivor data revealed nonlinearity, and therefore a simple first-order linear model was not appropriate to model the bacterial death-time relationship. Curves for some treatments had a shoulder and a small degree of tailing. The most common approach to describe microbial inactivation by thermal processing is to assume first-order kinetics. However, a first-order kinetics model is not compatible with curvature, a shoulder, or tailing of the survival curve (41). The first-order approach to thermobacteriology assumes that each microorganism has the same probability of dying (40). In the present study, because antimicrobial agents were applied on the bologna surface before the samples were exposed to heat the population of L. monocytogenes at the beginning of heating could consist of cells with various sensitivities to heat, leading to a nonlinear curve due to these differences in heat sensitivities. In reality, any bacterial population will consist of cells with different levels of heat resistance (40); however, the application of an antimicrobial may accentuate variation in the population.

The model that best fit the survivor curves obtained for 62.5 and 65°C was based on the Weibull distribution...
Two parameters of this model, $b$ and $n$, are scale and shape parameters, respectively. The estimates obtained for these parameters are given in Table 3. An $n$ value of $<1$ corresponds to a concave upward survival curve, $n > 1$ corresponds to a concave downward curve, and $n$ equal to 1 corresponds to a straight line. In the present study, all of the treatments yielded a value of $n < 1$, indicating an upward concavity of the curve. However, values of the estimate of $n$ in all cases were close to 1 (0.75 to 0.95), indicating that the upward concavity was minimal. Upward concavity can be interpreted as evidence that weak or sensitive members of the population are destroyed at a relatively fast rate, leaving behind survivors of higher resistance (25).

But in this study, the nisin and nisin-lysozyme treatments reduced the $L.\ monocytogenes$ population to below detection levels, indicating that there were only a small number of cells with higher heat resistance. This conclusion also is supported by the fact that there was no evidence of tailing, which would indicate the presence of heat-resistant survivors at the end of the pasteurization. Masschalck et al. (15) reported that a combination of nisin and lysozyme was helpful for reducing the tailing of survivor curves for high-pressure-treated bacterial populations because of the ability of this combination to reduce the fraction of cells that survive the treatment compared with the use of nisin or lysozyme alone. The parameter $b$ did not show any systematic pattern among treatments or between the two pasteurization temperatures. According to Mafart et al. (12), parameter $b$ has little significance. Rajan et al. (28) used the Weibull model to fit the survivor curves while studying the inactivation of $Bacillus\ stearothermophilus$ spores in egg patties treated by pressure-assisted thermal processing and observed that parameter $b$ of the Weibull model increased for a treatment that yielded a greater log reduction. At 65°C, all of the antimicrobial treatments yielded $b$ values more than twice that of the control treatment, reflecting the greater log reduction of antimicrobial-treated samples.

A modified log-logistic model was the best fit for the survivor curves at 60°C (Fig. 3). The parameter estimates obtained for this model are given in Table 3. The values of $\sigma$, which is the maximum rate of inactivation (maximum slope of inactivation curve), and $\tau$, which is the log time to the maximum rate of inactivation, did not show any obvious pattern among antimicrobial treatments. Stephens et al. (34) reported a linear increase in $\tau$ with decreases in temperature. However, in the present study the model was fitted to curves obtained during heating at a constant temperature (60°C). The Weibull model fits data that shoulder or tail but not data that display both upper and lower asymptotes. The log-logistic model does fit asymptotic data, which were obtained during the experiments conducted at 60°C.
FIGURE 2. Survival data for *Listeria monocytogenes* fitted with a Weibull model for various treatments at 65°C. Data are given for three replicates used in the study.

Even though two models used in this study fit the bacterial heat inactivation very closely, the parameters of these models did not directly give a clear indication of the inactivation kinetics. The influence of antimicrobial treatments on the parameters of the models was not conclusive. A measure of thermal processing that would be more useful to the industry is the log reduction of the target organism achieved by the process and the time needed to achieve a targeted log reduction. This point was also raised during the second research summit of the Institute of Food Tech-

### TABLE 3. Model parameters of Weibull and log-logistic models for antimicrobial treatments at different temperatures

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Treatment</th>
<th>Weibull</th>
<th>Log logistic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$b$</td>
<td>$n$</td>
</tr>
<tr>
<td>60</td>
<td>Control</td>
<td>2.987</td>
<td>2.582</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>2.545</td>
<td>2.984</td>
</tr>
<tr>
<td></td>
<td>Nisin</td>
<td>2.437</td>
<td>2.698</td>
</tr>
<tr>
<td></td>
<td>Nisin-lysozyme</td>
<td>6.827</td>
<td>2.433</td>
</tr>
<tr>
<td>62.5</td>
<td>Control</td>
<td>0.067</td>
<td>0.815</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>0.038</td>
<td>0.942</td>
</tr>
<tr>
<td></td>
<td>Nisin</td>
<td>0.066</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td>Nisin-lysozyme</td>
<td>0.042</td>
<td>0.959</td>
</tr>
<tr>
<td>65</td>
<td>Control</td>
<td>0.074</td>
<td>0.951</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>0.172</td>
<td>0.752</td>
</tr>
<tr>
<td></td>
<td>Nisin</td>
<td>0.168</td>
<td>0.788</td>
</tr>
<tr>
<td></td>
<td>Nisin-lysozyme</td>
<td>0.174</td>
<td>0.819</td>
</tr>
</tbody>
</table>

* Control, no antimicrobial added; lysozyme, 0.5 AU/cm² (80 AU/ml); nisin, 31.25 AU/cm² (5,000 AU/ml); nisin-lysozyme, 31.75 AU/cm² (5,080 AU/ml).
nologists to advance the understanding of microbial inactivation kinetics and models for non-log-linear survivor curves. It was suggested that “the performance of food preservation processes should be communicated in terms of the number of log cycles of reduction that the process is expected to deliver for the microorganism of concern rather than the D-value” (8). Therefore, by using the estimated parameters in the equation of the specific model and substituting the required value for the decimal reduction ratio in the equation, the time required for specific log reduction was calculated. When survivor curves are linear, the estimate of time of first decimal reduction allows comparison of microbial resistance because the inactivation rate is constant and independent of the pasteurization time (41). Because the survivor curves obtained in this study were not linear, the values of time of first decimal reduction are not suitable for comparing the effects of various antimicrobial treatments on the heat resistance of the organism. A more useful measure would be the estimated time required for a targeted log reduction or required to completely eliminate or reduce the *Listeria* population to a very low level. Because the curves were nonlinear, extrapolating the targeted log reduction beyond the maximum reduction obtained in this experiment would not be ideal. Therefore, considering the flexibility of both the models used in this study, the time required for a 4-log reduction in *L. monocytogenes* population was calculated (Table 4). The data obtained at 62.5 and 60°C were adequate to accurately determine 5- to 6-log reductions using the Weibull model parameters, but at 60°C the data could not be accurately extrapolated beyond a 4-log reduction because of the asymptotic nature of the logistic curve (lower asymptote). At 60°C, none of the antimicrobial treatments resulted in a significant difference in the time required to achieve a 4-log reduction compared with the control sample. At 62.5°C, nisin and nisin-lysozyme treatments resulted in a significant reduction (P < 0.05) in the time compared with the control and lysozyme treatments. The time required for a 4-log reduction was 149.07, 138.15, 123.63, and 115.49 s for control, lysozyme, nisin, and nisin-lysozyme treatments, respectively. The nisin-lysozyme treatment required about 35 s less than the control treatment to achieve a 4-log reduction at 62.5°C. The time required for a 4-log reduction in *L. monocytogenes* population at 65°C was 66.74, 65.47, 55.48, and 45.79 s for control, lysozyme, nisin, and nisin-lysozyme treatments, respectively. Nisin and nisin-lysozyme treatments resulted in a reduction (P < 0.05) in the time compared with the control and lysozyme treatments. Nisin-
Lysozyme treatment required about 21 s less than the control treatment to achieve the targeted reduction at 65°C. At 62.5 and 65°C, the time required for a 5-log reduction followed a trend similar to that for the 4-log reduction for the antimicrobial treatments (data not shown).

The estimated times required for 4-log reductions at 62.5 and 65°C indicate that presurface application of nisin and the nisin-lysozyme combination with in-package pasteurization was effective in reducing the time required for a targeted log reduction in pathogen populations. With higher concentrations of the antimicrobial agents than those used in this study, this effect may be more pronounced. In the present study, the concentrations of the antimicrobial agents used were optimized (reduced) to obtain log reduction points in the same range as that in the control sample to allow meaningful comparisons. At 60°C, the lowest temperature used in the study, the time estimates obtained for any of the antimicrobial treatments were not different (P > 0.05) from those for the control samples, possibly because the synergy of these antimicrobials with heat was less pronounced at the lower temperature.

In previous studies where a first-order kinetics model was fitted to obtain decimal reduction times (D-values), researchers have reported the ability of nisin and other antimicrobial agents to reduce D-values. Wandlell et al. (42) found that the addition of 2,000 IU/ml nisin in skim milk lowered the D-value from 16.0 (control) to 13.8 s. Knight et al. (10) reported that nisin at a concentration of 10 μg/ml (40 IU/ml) significantly reduced the D-value of L. monocytogenes in liquid whole egg at low pasteurization temperatures (<58°C). From a practical point of view, results from these two studies also suggest that nisin treatment can significantly reduce the time required for a targeted log reduction.

In the present study, presurface application of nisin and a combination of nisin and lysozyme (1:5) were effective in reducing the time required for a targeted log reduction in L. monocytogenes populations on the RTE bologna surface at 62.5 and 65°C. Another advantage of combining heat and an antimicrobial agent is the possibility of reducing the concentration of the antimicrobial agent required to achieve adequate lethality compared with concentration required to attain the same lethality when the agent is used without heat. Furthermore, the most aggressive USDA post- in-package pasteurization lethality option includes the use of an inhibitor for L. monocytogenes outgrowth during storage, and application of nisin or nisin-lysozyme may meet this criterion. Results from this study may be useful to the industry for developing a cost-effective in-package pasteurization process. Shorter pasteurization times also will help preserve the desirable qualities of the food product. Future studies are needed to determine the antilisterial activity of this combined method on various RTE poultry products with different compositions, thicknesses, and other characteristics.

**ACKNOWLEDGMENTS**

The authors thank Danisco and Cryovac Division of Sealed Air Corporation for donating supplies used in these experiments. This research was partially funded by the U.S. Poultry and Egg Association.

**REFERENCES**


**TABLE 4. Time required for 4-log CFU/cm² reduction in Listeria monocytogenes population on the surface of turkey bologna subjected to in-package pasteurization**

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Treatment</th>
<th>Mean (±SD) time to 4-log reduction (s)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Control</td>
<td>497.29 ± 22.45 A</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>462.22 ± 12.34 A</td>
</tr>
<tr>
<td></td>
<td>Nisin</td>
<td>519.10 ± 12.43 A</td>
</tr>
<tr>
<td></td>
<td>Nisin-lysozyme</td>
<td>515.18 ± 47.68 A</td>
</tr>
<tr>
<td>62.5</td>
<td>Control</td>
<td>149.10 ± 9.68 A</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>138.15 ± 10.10 A</td>
</tr>
<tr>
<td></td>
<td>Nisin</td>
<td>123.63 ± 4.86 b</td>
</tr>
<tr>
<td></td>
<td>Nisin-lysozyme</td>
<td>115.49 ± 6.75 b</td>
</tr>
<tr>
<td>65</td>
<td>Control</td>
<td>66.74 ± 3.30 A</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>65.47 ± 4.75 A</td>
</tr>
<tr>
<td></td>
<td>Nisin</td>
<td>55.48 ± 2.69 b</td>
</tr>
<tr>
<td></td>
<td>Nisin-lysozyme</td>
<td>45.79 ± 3.34 b</td>
</tr>
</tbody>
</table>

a Control, no antimicrobial added; lysozyme, 0.5 AU/cm² (80 AU/ml); nisin, 31.25 AU/cm² (5,000 AU/ml); nisin-lysozyme, 31.75 AU/cm² (5,080 AU/ml).

b Within each temperature, means with different letters are significantly different (P < 0.05).


