Nisin affects the growth of *Listeria monocytogenes* on ready-to-eat turkey ham stored at four degrees Celsius for sixty-three days

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**ABSTRACT** The objectives of this study were to determine pH, anti-*Listeria* and general antimicrobial properties of nisin on ready-to-eat vacuum-packaged diced turkey ham inoculated with *Listeria monocytogenes*, and the usage level that would exert maximum antimicrobial effect during 63 d of storage. Ready-to-eat diced turkey ham was inoculated with a 5-strain *L. monocytogenes* cocktail; treated with 0.2, 0.3, 0.4, and 0.5% nisin treatment solutions; vacuum-packaged; stored at 4 ± 1°C for 63 d; and analyzed at 1-wk intervals for total aerobic counts, pH, *L. monocytogenes*, and lactic acid bacteria. Antimicrobial effectiveness of nisin increased as concentration increased from 0.2 to 0.5%. Aerobic plate counts for 0.4 and 0.5% nisin were lower (*P* < 0.05) than negative and positive controls. All nisin treatments resulted in 4 log reductions (*P* < 0.05) in *L. monocytogenes* when compared with the positive control on d 0. Four log reductions were also observed on d 7 for 0.4% nisin treatment and d 7 and 14 for 0.5% nisin treatment when compared with the positive control. *Listeria monocytogenes* counts decreased from 4.97 log cfu/g on d 0 and remained less than 2 log cfu/g through 63 d of storage for the 0.5% nisin treatment. Lactic acid bacteria counts were lower (*P* < 0.05) for 0.5% nisin treatment when compared with positive and negative controls from 28 through 63 d. Except for d 56 and 63, pH was similar (*P* < 0.05) for all treatments. This study revealed that nisin could be used for post-processing intervention to control *L. monocytogenes* in ready-to-eat poultry products.

**Key words:** nisin, bacteriocin, turkey ham, ready-to-eat product, *Listeria monocytogenes*

INTRODUCTION

*Listeria monocytogenes* is the causative agent of listeriosis and has resulted in numerous major foodborne outbreaks worldwide (Gombas et al., 2003). The ability of *L. monocytogenes* to grow at temperatures ranging from 0 to 45°C (Barbosa et al., 1994), tolerate salt (Farber and Peterkin, 1991), and grow at a relatively low pH (Bell and Kyriakides, 2005) causes the bacteria to be difficult to control in food. Hygienic and sanitation practices applied in meat processing facilities are often insufficient to prevent contamination of processed meat products (Cox et al., 1989). *Listeria monocytogenes* is resistant to many food preservation methods and can increase to high numbers during refrigerated storage (Walker et al., 1990) and low oxygen tension (Lou and Yousef, 1999). Although the heat treatment (cooking) that ready-to-eat (RTE) meat and poultry products undergo eliminates the pathogen, recontamination may occur during postprocessing procedures such as peeling, slicing, and repackaging (Farber and Peterkin, 1999). Therefore, effective postprocessing antimicrobial interventions to inhibit growth of the pathogen are essential.

A novel approach to controlling *L. monocytogenes* in foods is the use of antimicrobial bacteriocins from lactic acid bacteria (Muriana, 1996). Nisin, a lanthionine-containing polypeptide produced by *Lactococcus lactis* ssp. *lactis* (Altena et al., 2000), is a bacteriocin with antimicrobial activity against *L. monocytogenes* (Bruno and Montville, 1993). The half-maximal lethal dose value was found to be similar to that of common salt (Montville and Bruno, 1994). Nisin is generally recognized as safe for use as a biopreservative in food systems. It is approved for use in meat and poultry products at 250 mg/kg in the finished product, 6.30 mg/kg in the finished product when used in casings, 5.0 mg/kg on cooked meat and poultry products, and 550 mg/kg of a blend of encapsulated nisin preparation (90.9%), rosemary extract (8.2%), and salt (0.9%) for frankfurt-
ers and other similar cooked meat and poultry sausages (US Food and Drug Administration, 2008).

Applications that have been considered or investigated for nisin in poultry and meat systems include addition of nisin-producing Lactococcus lactis ssp. lactis to meat systems in an attempt to produce nisin in situ (Abee et al., 1994), antimicrobial dipping solutions (Zhang and Mustapha, 1999; Ariyapitipun et al., 2000; Samelis et al., 2005), nisin-coated casings (Luchansky and Call, 2004), direct addition of nisin into meat formulations (Gill and Holley, 2000; Samelis et al., 2002), antibiotulinal agent for the partial replacement of nitrite in cooked meat systems (Abee et al., 1994), and use in canned meats as a means of reducing thermal processing time (Montville and Chen, 1998).

The mechanism of action for nisin is based on the disruption of the cytoplasmic cell membrane, as evidenced by the rapid efflux of small molecules from both whole cells and liposomes (Garcera et al., 1993; Abee et al., 1994; Winkowski et al., 1994). As a result, nisin depletes the proton motive force of sensitive cells and artificial liposomes (Gao et al., 1991; Bruno et al., 1992). Nisin acts through a multistep process, which includes binding of nisin to the cell, insertion into the membrane, and pore formation (Sahl, 1991; Garcera et al., 1993).

A major issue with the use of nisin in RTE poultry and meat products is determining the proper usage level and appropriate application method. This study proposes the addition of nisin treatment solutions to the RTE product during the final packaging step to ensure that the treatment solution is in direct contact with the ham and remains in the package throughout storage. The objectives of this study were to determine pH, anti-Listeria and general antimicrobial properties of nisin on RTE vacuum-packaged diced turkey ham inoculated with L. monocytogenes, and the usage level that would exert maximum antimicrobial effect during 63 d of storage.

**MATERIALS AND METHODS**

**Inoculum Cultivation, Storage, and Preparation**

Five reference strains of L. monocytogenes, 1/2a, 1/2b, 4b, Scott A, and 19115, were obtained from ABC Research Corporation in Gainesville, Florida, and used as the inoculum in this study. Initially, stock solutions were prepared by transferring each strain to test tubes containing 10 mL of tryptic soy broth (TSB, DF 0369-17-6, Difco Laboratories, Detroit, MI) using a flamed-sterilized 3-mm inoculation loop. The broth was incubated at 35°C for 24 h. After incubation, the cultures were poured into sterile 15-mL centrifuge tubes and centrifuged (Sorvall RC-5B, Du Pont Instruments, Newton, CT; Type SS-34 rotor, Sorvall Instruments, Newton, CT) at 2,988 × g (Science Gateway, 2009) for 10 min at 16°C. The supernatant was discarded and the pellets were resuspended in 10 mL of sterile 0.1% buffered peptone water (BPW, DF O1897-17-4, Difco Laboratories) and recenterifuged. The supernatant was discarded and the pellets were resuspended in 1 mL of 3% TSB with 30% glycerol in a 2-mL cryovial (03-374-2, Corning Inc., Corning, NY), stored at −45°C, and used as the stock culture for the inoculation studies.

Twenty-four hours before conducting the study, 1 tube of each of the individual strains was removed from the freezer and allowed to thaw at room temperature for 10 min. A loopful of the cultures from each strain was transferred and mixed in test tubes containing 10 mL of 3% TSB, vortexed, and incubated at 35°C for 24 h. Two consecutive 24-h transfers of the stock cultures were conducted to obtain a culture in which the cells were in the same physiological state. After incubation, each culture was centrifuged at 2,988 × g for 10 min at 16°C, washed with sterile BPW, resuspended in BPW, mixed to form the 5-strain inoculum, and serially diluted with BPW to concentrations of 10⁻¹ to 10⁻⁸. Preliminary work was conducted to determine the concentration of inoculum needed to yield 4 to 5 log cfu/g on the ham samples.

**Preparation of Nisin Solutions for Turkey Ham Samples**

Nisin solutions containing 0.2, 0.3, 0.4, and 0.5% nisin were prepared using Nisaplin (a commercial product containing 10⁶ IU of nisin/g, Danisco, Copenhagen, Denmark), 0.02 N food-grade HCl (7647-01-0, Fisher Scientific, Pittsburgh, PA), and salt (S9625-500G, Sigma Chemical, St. Louis, MO) based on the total formula weight. The HCl is used in meat systems primarily for ensuring that the nisin is in the solution before coming in contact with the complex meat system, which may interfere with the activity of nisin (Liu and Hansen, 1999; Rose et al., 1999).

**Inoculation and Treatment**

Commercially available turkey hams were purchased from a local supermarket as soon as the shipment arrived at the store and were used in this study. All hams purchased had sell-by dates of at least 60 d. The turkey hams were transported to the research laboratory on ice packs and stored in a walk-in cooler at 4 ± 1°C for no longer than 24 h before using. The hams were aseptically transferred from the vacuum-packaged bag to presterilized trays and were chopped into approximately 0.5-cm pieces as is typical for ham used in salads and sandwiches.

Approximately 3 kg of chopped turkey ham was placed on presterilized trays and was inoculated by spraying with a 10⁶ cfu/mL L. monocytogenes inoculum. Inoculated samples were left to stand at room temperature for 20 min to allow for bacterial attachment to ensure a
Nisin affects the growth of Listeria monocytogenes

A complete randomized block design was employed. A total of 240 samples were analyzed (i.e., duplicate samples, 6 treatments, 10 storage days, 2 trials). The general GLM program (PROC GLM) of SAS (Version 8.02, SAS Institute, Cary, NC) was employed to determine differences between trials, among treatments and storage days, and treatment × day interaction. Any significant differences were determined using SAS Tukey multiple range test procedure at a level of significance of α = 0.05.

RESULTS AND DISCUSSION

Aerobic Bacteria Analysis

In general, the APC revealed no evidence of poor sanitation or cross contamination (Table 1). Except for the positive control, all APC were less than 2.50 log cfu/g. The 0.4 and 0.5% nisin treatments resulted in lower (P < 0.05) APC when compared with the negative and positive controls. The 5 log cfu/g count for the positive control was indicative of the presence of the inoculum. The 0.4 and 0.5% nisin treatments resulted in 1.21 and 1.70 log reductions, respectively, when compared with the negative control and 3.97 and 4.40 log reductions, respectively, when compared with the positive control. The 0.2 and 0.3% nisin treatments resulted in less than 1 log reduction when compared with the negative control and 3.03 to 3.47 log reductions, respectively, when compared with the positive control.

pH Analysis

The pH values were similar (P > 0.05) for all treatments from d 0 to 49 (Table 2). On d 56, hams treated with 0.5% nisin had higher (P < 0.05) pH values when compared with all other treatments. On d 63, hams treated with 0.5% nisin had higher (P < 0.05) pH values than the negative and positive control hams. Except for hams treated with 0.3 and 0.5% nisin, the pH of all treatments decreased (P < 0.05) during storage. Although not always significant, the pH values decreased

### Table 1. Mean aerobic plate counts for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with Listeria monocytogenes, and analyzed before storage1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0 (log cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>2.29b</td>
</tr>
<tr>
<td>Positive control</td>
<td>5.04a</td>
</tr>
<tr>
<td>0.2% nisin</td>
<td>1.91bc</td>
</tr>
<tr>
<td>0.3% nisin</td>
<td>1.57cde</td>
</tr>
<tr>
<td>0.4% nisin</td>
<td>1.07def</td>
</tr>
<tr>
<td>0.5% nisin</td>
<td>0.58d</td>
</tr>
</tbody>
</table>

1Means within a column lacking a common superscript differ (P < 0.05).

1Each mean value represents 8 individual measurements.
as the concentration of nisin decreased. This may be attributed to the production of numerous compounds such as acidic metabolites and carbonic acid that may decrease pH (Doyle et al., 2001).

**L. monocytogenes Analysis**

*L. monocytogenes* counts remained at 4.14 to 4.97 log cfu/g through 63 d of storage for the positive control, which confirmed that the desired *L. monocytogenes* inoculum concentration of 4 log cfu/g was achieved (Table 3). In general, all nisin treatments resulted in reduced (*P* < 0.05) *L. monocytogenes* counts when compared with the positive control on d 0, 7, and 49. No *L. monocytogenes* was isolated on the negative controls. On d 0, all nisin treatments resulted in 4 log reductions (*P* < 0.05) in *L. monocytogenes* when compared with the positive control. The 4 log reductions were also observed on d 7 for 0.4% nisin treatment and d 7 and 14 for the 0.5% nisin treatment (*P* < 0.05) when compared with the positive control. Two log reductions in *L. monocytogenes* were observed 21 through 63 d for samples treated with 0.5% nisin. The treatment × day interaction (*P* < 0.05) was attributed primarily to the increase (*P* < 0.05) in *L. monocytogenes* counts on d 42 through 63 for the 0.2% nisin treatment. Except for the 0.2% nisin treatment on d 42 through 63, no significant increase in *L. monocytogenes* counts occurred for hams treated with nisin through 63 d of storage. The 0.5% treatment demonstrated an extended lag phase through 63 d of storage, in which *L. monocytogenes* counts remained less than 1.96 log cfu/g. The reduction in *L. monocytogenes* counts on d 49 for all hams treated with nisin when compared with the positive control suggested that a continual antimicrobial effect was being exerted by the nisin treatments.

**Lactic Acid Bacteria Analysis**

Lactic acid bacteria populations increased as storage time increased for all treatments (Table 4). On d 0 and 49, lactic acid bacteria counts were significantly lower (*P* < 0.05) for all nisin treatments when compared with the positive control. Except for 7, 14, and 21, hams treated with 0.5% nisin had lower (*P* < 0.05) lactic acid bacteria counts when compared with the positive and negative controls. Lactic acid bacteria counts were also lower (*P* < 0.05) for hams treated with 0.3% nisin (*P* < 0.05) when compared with positive and negative controls on d 56. This observation revealed the antimicrobial properties of nisin against lactic acid bacteria. It was discussed earlier that the pH values increased as the concentration of nisin increased to 0.5%. The data suggested that the shelf life of vacuum-packaged cured RTE poultry products may be extended with the use of 0.5% nisin by suppression of the growth of lactic acid bacteria.

In conclusion, this study revealed that nisin can be successfully incorporated into RTE turkey ham to control *L. monocytogenes* and lactic acid bacteria. The an-

### Table 2. pH measurements for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *Listeria monocytogenes*, and stored at 4 ± 1°C for 63 d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>56</th>
<th>63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>6.24±a</td>
<td>5.76±y</td>
<td>4.97±z</td>
<td>4.87±z</td>
<td>4.86±y</td>
<td>4.81±y</td>
<td>4.79±y</td>
<td>4.67±y</td>
<td>4.86±y</td>
<td>4.82±y</td>
</tr>
<tr>
<td>Positive</td>
<td>6.14±x</td>
<td>5.07±y</td>
<td>4.85±y</td>
<td>4.79±y</td>
<td>4.78±y</td>
<td>4.82±y</td>
<td>4.77±y</td>
<td>4.94±y</td>
<td>4.74±y</td>
<td>4.77±y</td>
</tr>
<tr>
<td>0.2% nisin</td>
<td>6.21±z</td>
<td>5.67±y</td>
<td>5.46±y</td>
<td>5.29±y</td>
<td>5.19±y</td>
<td>5.00±y</td>
<td>5.02±y</td>
<td>5.13±y</td>
<td>5.01±y</td>
<td>4.86±y</td>
</tr>
<tr>
<td>0.3% nisin</td>
<td>6.22±a</td>
<td>6.10±y</td>
<td>5.72±y</td>
<td>5.43±y</td>
<td>5.41±y</td>
<td>5.40±y</td>
<td>5.29±y</td>
<td>5.10±y</td>
<td>5.24±y</td>
<td></td>
</tr>
<tr>
<td>0.4% nisin</td>
<td>6.26±z</td>
<td>5.85±y</td>
<td>5.56±y</td>
<td>5.41±y</td>
<td>5.06±y</td>
<td>5.15±y</td>
<td>4.90±y</td>
<td>4.76±y</td>
<td>4.87±y</td>
<td>5.12±y</td>
</tr>
<tr>
<td>0.5% nisin</td>
<td>6.21±z</td>
<td>6.14±y</td>
<td>6.14±y</td>
<td>5.89±y</td>
<td>5.58±y</td>
<td>5.70±y</td>
<td>5.67±y</td>
<td>5.80±y</td>
<td>5.83±y</td>
<td>5.83±y</td>
</tr>
</tbody>
</table>

*Means within a column lacking a common superscript differ (*P* < 0.05).

### Table 3. *Listeria monocytogenes* counts (log cfu/g) for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *L. monocytogenes*, and stored at 4 ± 1°C for 63 d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>56</th>
<th>63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0.00±y</td>
<td>0.00±y</td>
<td>0.00±y</td>
<td>0.00±y</td>
<td>0.00±y</td>
<td>0.00±y</td>
<td>0.00±y</td>
<td>0.00±y</td>
<td>0.00±y</td>
<td>0.00±y</td>
</tr>
<tr>
<td>Positive</td>
<td>4.97±y</td>
<td>4.92±y</td>
<td>4.95±y</td>
<td>4.30±y</td>
<td>4.23±y</td>
<td>4.32±y</td>
<td>4.18±y</td>
<td>4.14±y</td>
<td>4.55±y</td>
<td>4.31±y</td>
</tr>
<tr>
<td>0.2% nisin</td>
<td>0.97±y</td>
<td>2.12±y</td>
<td>3.22±y</td>
<td>2.47±y</td>
<td>2.94±y</td>
<td>3.35±y</td>
<td>3.57±y</td>
<td>2.97±y</td>
<td>3.61±y</td>
<td>3.52±y</td>
</tr>
<tr>
<td>0.3% nisin</td>
<td>0.60±y</td>
<td>1.50±y</td>
<td>1.96±y</td>
<td>2.16±y</td>
<td>2.95±y</td>
<td>2.62±y</td>
<td>2.57±y</td>
<td>1.83±y</td>
<td>2.99±y</td>
<td>3.10±y</td>
</tr>
<tr>
<td>0.4% nisin</td>
<td>0.60±y</td>
<td>0.85±y</td>
<td>2.29±y</td>
<td>1.32±y</td>
<td>2.50±y</td>
<td>2.96±y</td>
<td>3.00±y</td>
<td>2.09±y</td>
<td>3.02±y</td>
<td>2.82±y</td>
</tr>
<tr>
<td>0.5% nisin</td>
<td>0.42±y</td>
<td>0.89±y</td>
<td>0.17±y</td>
<td>1.66±y</td>
<td>1.90±y</td>
<td>1.95±y</td>
<td>1.67±y</td>
<td>1.01±y</td>
<td>1.89±y</td>
<td>1.51±y</td>
</tr>
</tbody>
</table>

*Means within a column lacking a common superscript differ (*P* < 0.05).

<sup>1</sup>Each mean value represents 8 individual measurements.
timicrobial effectiveness of nisin increased as its concentration increased from 0.2 to 0.5% with the most effective antimicrobial level being 0.5%. The data revealed that 0.2, 0.3, and 0.4% nisin treatments resulted in lower (P < 0.05) L. monocytogenes when compared with the positive control initially (d 0) and for 1 wk (7 d) and on d 49. In comparison, the 0.5% nisin treatment resulted in lower (P < 0.05) L. monocytogenes counts initially (d 0) and for 2 wk (14 d) and on d 49 and 63. Although the counts on d 21, 28, 35, 42, and 56 were not significantly lower (P > 0.05) than the positive control, they were at least 2 log less than the positive control. The data for pH revealed that 0.5% nisin was effective in increasing pH of the ham and simultaneously decreasing lactic acid bacteria counts. This study also revealed that the 0.5% nisin treatment exerted maximum antimicrobial effects on L. monocytogenes and lactic acid bacteria during 63 d of storage. Listeria monocytogenes counts decreased from 4.97 log cfu/g on d 0 and remained less than 2 log cfu/g through 63 d of storage for the 0.5% nisin treatment.

**REFERENCES**


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**Table 4. Lactic acid bacteria counts (log cfu/g) for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with Listeria monocytogenes, and stored at 4 ± 1°C for 63 d**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>56</th>
<th>63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>3.25b premium</td>
<td>4.74a premium</td>
<td>5.66x premium</td>
<td>6.86x premium</td>
<td>7.10x premium</td>
<td>6.84x profit</td>
<td>6.66x profit</td>
<td>6.71x premium</td>
<td>6.62x profit</td>
<td>6.66x profit</td>
</tr>
<tr>
<td>Positive</td>
<td>4.27x premium</td>
<td>5.49x profit</td>
<td>5.90x premium</td>
<td>6.79x premium</td>
<td>6.61x premium</td>
<td>6.74x premium</td>
<td>6.54x premium</td>
<td>6.47x premium</td>
<td>6.53x premium</td>
<td>6.71x premium</td>
</tr>
<tr>
<td>0.2% nisin</td>
<td>1.55y premium</td>
<td>3.60x profit</td>
<td>4.18x profit</td>
<td>4.65x profit</td>
<td>4.43x profit</td>
<td>5.28x profit</td>
<td>5.43x profit</td>
<td>5.57x profit</td>
<td>5.65x profit</td>
<td>5.12x profit</td>
</tr>
<tr>
<td>0.3% nisin</td>
<td>1.99x profit</td>
<td>2.44x profit</td>
<td>2.79x profit</td>
<td>3.46x profit</td>
<td>3.94x profit</td>
<td>4.95x profit</td>
<td>4.96x profit</td>
<td>4.31x profit</td>
<td>4.52x profit</td>
<td>4.94x profit</td>
</tr>
<tr>
<td>0.4% nisin</td>
<td>1.28x profit</td>
<td>2.73x premium</td>
<td>3.43x profit</td>
<td>3.86x profit</td>
<td>4.80x premium</td>
<td>5.41x profit</td>
<td>5.18x profit</td>
<td>5.66x profit</td>
<td>5.29x profit</td>
<td>5.11x profit</td>
</tr>
<tr>
<td>0.5% nisin</td>
<td>1.32x profit</td>
<td>1.02x profit</td>
<td>1.59x profit</td>
<td>3.07x profit</td>
<td>3.13x profit</td>
<td>4.20x profit</td>
<td>4.19x profit</td>
<td>4.23x profit</td>
<td>3.48x profit</td>
<td>3.00x profit</td>
</tr>
</tbody>
</table>

*Means within a column lacking a common superscript differ (P < 0.05).

*Means within a row lacking a common superscript differ (P < 0.05).

1Each mean value represents 8 individual measurements.


