Inhibition of *Listeria monocytogenes* Using Nisin with Grape Seed Extract on Turkey Frankfurters Stored at 4 and 10°C

T. SIVAROOBAN, N. S. HETTIARACHCHY,* AND M. G. JOHNSON

Department of Food Science, University of Arkansas, 2650 North Young Avenue, Fayetteville, Arkansas 72704, USA

MS 06-151: Received 14 March 2006/Accepted 22 July 2006

**ABSTRACT**

Recontamination of cooked ready-to-eat (RTE) chicken and beef products with *Listeria monocytogenes* has been a major safety concern. Natural antimicrobials in combinations can be an alternative approach for controlling *L. monocytogenes*. Therefore, the objectives of this study were to evaluate the inhibitory activities against *L. monocytogenes* of nisin (6,400 IU/ml), grape seed extract (GSE; 1%), and the combination of nisin and GSE both in tryptic soy broth with 0.6% yeast extract (TSBYE) and on the surface of full-fat turkey frankfurters. TSBYE was incubated at 37°C for 72 h and turkey frankfurters at 4 or 10°C for 28 days. Inocula were 6.7 or 5 log CFU per ml or g for TSBYE or frankfurters, respectively. After 72 h in TSBYE, nisin alone did not show any inhibitory activity against *L. monocytogenes*. The combination of nisin and GSE gave the greatest inhibitory activity in both TSBYE and on turkey frankfurters with reductions of *L. monocytogenes* populations to undetectable levels after 15 h and 21 days, respectively. This combination of two natural antimicrobials has the potential to control the growth and recontamination of *L. monocytogenes* on RTE meat products.

The issue of recontamination of cooked ready-to-eat (RTE) poultry meat with *Listeria monocytogenes* has posed a major health concern to the general public and was re-addressed in a recent Food Safety and Inspection Services directive published in the Federal Register requesting a new standard for processing of these meat products (25). In 2005, 300,424 pounds of RTE meat products were recalled for possible *L. monocytogenes* contamination (9). *L. monocytogenes* can survive under normally limiting extreme physicochemical conditions such as refrigeration temperatures (as low as 1°C), low pH, high salt concentration, and high temperatures (13). Currently, there is a significant interest in using natural antimicrobial combinations against foodborne pathogens. The application of these natural antimicrobial combinations can induce sublethal stresses on pathogens, thereby helping prevent the growth of recontaminating pathogens on food products. This multiple hurdle approach has great potential in food preservation and allows use of lower concentrations of antimicrobials.

Several plant-derived compounds including phenolics, terpenoids, essential oils, alkaloids, lectins, polypeptides, and polyacetylenes possess effective antimicrobial activities. These compounds act through various mechanisms such as membrane disruption, adhesion binding, protein and cell wall binding, enzyme inactivation, and intercalation into the cell wall and/or DNA to inhibit pathogens (5). Studies have demonstrated the effectiveness of plant extracts as antimicrobials (7); however, only a few plant extracts have been shown to be effective in food systems as opposed to laboratory model systems (15, 23). Plant extracts, including rosemary (19), grape seed extract ActiVin (1), clove (10), and hop extracts (17), have been shown to be effective against *L. monocytogenes* in meat systems.

Nisin, a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, has been recognized as a safe biological food preservative (18). Nisin is effective in controlling a wide range of gram-positive bacteria, including *L. monocytogenes* (16, 22). However, the use of nisin alone in food products may encourage the emergence of nisin-resistant mutants (6). To avoid this, nisin is often combined with other preservation techniques as a component of a multiple hurdle approach whereby the combination of several physical and/or chemical preservation methods can be used to control foodborne pathogens (4). However, there is a limited understanding of the inhibitory activities of the nisin plus natural extract combinations against *L. monocytogenes* on RTE meat products. Therefore, the objectives of this study were (i) to evaluate the inhibitory effect of GSE against *L. monocytogenes* in a laboratory medium at 37°C, (ii) to determine the combined inhibitory effect of nisin with GSE in a laboratory medium at 37°C, and (iii) to evaluate the potential application of combined nisin with GSE against surface inoculated *L. monocytogenes* on frankfurters stored at 4 or 10°C up to 28 days.

**MATERIALS AND METHODS**

Evaluating inhibitory activity of GSE against *L. monocytogenes* at 37°C in a model system. The *L. monocytogenes* V7 serotype (1/2a) FDA, was obtained from Dr. Johnson’s laboratory (Center for Food Safety–IFSE, University of Arkansas). A loop of *L. monocytogenes* was taken from a frozen stock culture stored...
TABLE 1. Inhibitory effect of varying concentrations of grape seed extract (GSE) against Listeria monocytogenes in a model system for 24 h at 37°C.

<table>
<thead>
<tr>
<th>Dosage of GSE (mg/ml)</th>
<th>L. monocytogenes (log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>9.0 ± 0.01 A</td>
</tr>
<tr>
<td>10</td>
<td>4.6 ± 0.09 B</td>
</tr>
<tr>
<td>20</td>
<td>4.3 ± 0.11 C</td>
</tr>
<tr>
<td>30</td>
<td>3.4 ± 0.14 D</td>
</tr>
<tr>
<td>40</td>
<td>3.0 ± 0.17 E</td>
</tr>
</tbody>
</table>

*Condition: TSBYE medium, the initial inoculum was 6.70 log CFU/ml, 37°C for 24 h incubation. A platform shaker was used at 250 rpm. Plating medium: tryptic soy agar with 0.6% yeast extract.

*Statistical analysis: values are means of three determinations.*

General linear model procedure with analysis of variance was conducted using the Statistical Analysis System (SAS 8.2, SAS Institute, Inc., Cary, N.C.). The least significant difference procedure (Student’s t test) was used to compare means and significant differences among the treatments. Column values with the same letter were not significantly different at \( P < 0.05 \). Minimum detection limit was 10 CFU/ml.

Evaluating the inhibitory activity of GSE, nisin, and the combination of nisin with GSE against L. monocytogenes at 37°C in a model system. Cell pellets were prepared as described above. One-milliliter aliquots of TSBYE solution, containing GSE (1%), nisin (6,400 IU/mL) (Nisaplin, Alpin and Barrett Ltd., Beaminster, Dorset, UK), and the combination of nisin and GSE, were added to the bacterial cell pellets in Eppendorf centrifuge tubes and incubated for 72 h at 37°C in 1-ml volumes of TSBYE on a rotary platform shaker (250 rpm). A control sample that consisted of 1 ml of TSBYE was also included. All samples were made in triplicate. Following incubation, L. monocytogenes cells were enumerated at 6 h intervals as described above.

Evaluating the inhibitory effects of GSE, nisin, and the combination of nisin with GSE in meat system. For the preparation of turkey frankfurter samples, full-fat (fat content 21%) commercial turkey frankfurters lacking sodium lactate and sodium diacetate were purchased from a local supermarket, diced into cubes (1 cm³), and packaged (20 pieces per bag) in Whirl-Pak bags (4 oz, 120-ml capacity, 7.5 cm by 18.5 cm; National Account Service Company LLC [NASCO], Fort Atkinson, Wis.). To eliminate the effects of indigenous microflora, these meat cubes were irradiated with a 30-kGy dose using a linear electron accelerator (Texas A&M University, College Station) and kept frozen until used.

To evaluate the potential use of these antimicrobials on post-processing contaminations, the meat samples were first dipped in antimicrobial solutions and then challenged with L. monocytogenes. Cubes of irradiated meat samples (1 cm³) were individually dipped for 1 min into the 200 ml of prepared extract solutions, containing GSE, nisin, or the combination of nisin with GSE. The positive control, distilled water, containing no antimicrobial agents, was also included. A total of 168 (triplicates of 4 treatments × 2 temperatures × 7 days) sample meat cubes were treated and allowed to drip dry under a laminar hood for 1 h. The dried meat cubes were then dipped into the culture broth for 30 sec. The same method previously explained was used to prepare inoculum, containing approximately 5 log CFU/ml of L. monocytogenes. After inoculation, the meat cubes were allowed to drip dry of excess inoculum under a laminar hood and were then packaged individually in Whirl-Pak bags and stored at 4 or 10°C.

Bacterial counts during storage on days 0, 7, 14, 21, and 28. On days 0, 7, 14, 21, and 28 triplicate pieces of meat were removed and stomached for 2 min and decimally diluted in PBS at pH 7.0. Dilutions were stabilized in duplicate on TSAYE plates and kept counted after incubation at 37°C for 48 h.
TABLE 2. Combined effect of nisin and grape seed extract (GSE) on the inhibition of L. monocytogenes on turkey frankfurters stored at 4°C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (day): 0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lm control</td>
<td>5.0 ± 0.07 B</td>
<td>5.8 ± 0.06 A</td>
<td>5.5 ± 0.09 A</td>
<td>6.7 ± 0.06 A</td>
<td>7.1 ± 0.16 A</td>
</tr>
<tr>
<td>GSE</td>
<td>5.1 ± 0.08 AB</td>
<td>5.5 ± 0.13 B</td>
<td>5.5 ± 0.10 A</td>
<td>6.3 ± 0.13 B</td>
<td>7.0 ± 0.05 A</td>
</tr>
<tr>
<td>Nisin</td>
<td>5.2 ± 0.12 A</td>
<td>3.1 ± 0.17 C</td>
<td>5.1 ± 0.15 B</td>
<td>6.0 ± 0.31 C</td>
<td>6.3 ± 0.12 B</td>
</tr>
<tr>
<td>Nisin + GSE</td>
<td>5.1 ± 0.05 AB</td>
<td>3.1 ± 0.16 C</td>
<td>2.5 ± 0.19 C</td>
<td>0.0 ± 0.00 d</td>
<td>0.0 ± 0.00 c</td>
</tr>
</tbody>
</table>

a Lm control, inoculation of L. monocytogenes on turkey frankfurters in which dipping solution contained no antimicrobial agents. Turkey frankfurters were dipped in a deionized water solution containing 1% GSE (GSE), 6,400 IU nisin (nisin), or in a solution containing a combination of 6,400 IU nisin and 1% GSE (nisin + GSE).

b All means are measurements of three separate experiments in duplicate. Values are means of three determinations. Means within a column followed by the same letter(s) are not significantly different (P < 0.05). Minimum detection limit was 100 CFU/g.

RESULTS AND DISCUSSION

Antimicrobial activities of GSE. Table 1 shows GSE at 10, 20, 30, or 40 mg/ml in TSBYE limited growth and caused an apparent inhibition of cells to yield counts of 4.6, 4.3, 3.4, and 3.0 log CFU/ml, respectively, at 24 h. During this incubation time, the control inoculum had increased from 6.7 to 9.0 log CFU/ml.

Inhibitory effects of GSE, nisin, or combination of nisin with GSE against L. monocytogenes in TSBYE at 37°C. When the test was extended to 72 h, the 1% GSE plus 6,400 IU nisin treatment combination caused an apparent reduction of survivor cell levels below the minimum level of detection (i.e., 10 CFU/ml). The GSE treatment alone gave a reduction from of 6.5 to 4.5 log CFU/ml by 12 to 18 h but no subsequent further reductions were observed. The nisin treatment alone gave an initial reduction of cell numbers of about 3 log CFU/ml by 12 h but cell numbers rebounded by 18 to 24 h, reaching the same numbers as the untreated control of about 9 log CFU/ml (Fig. 1).

The GSE plus nisin combination gave enhanced inhibition by reducing the L. monocytogenes population to undetectable levels in the TSBYE at 37°C. It is likely that the presence of GSE with nisin prevents the growth of nisin-resistant survivors in the subpopulation. Both nisin and GSE are believed to act upon the cytoplasmic membrane of the cell. A similar synergistic inhibitory activity against L. monocytogenes (8, 20) was recently reported when nisin was combined with carvacrol or the essential oil thymol.

Inhibitory effects of nisin and GSE, and the combined effect of nisin with GSE against L. monocytogenes on turkey frankfurters stored at 4 and 10°C. Tables 2 and 3 show the effect of nisin, GSE, and the combination effect of nisin and GSE against L. monocytogenes on full-fat turkey frankfurters at 4 or 10°C over 28 days, respectively. For 4°C studies, the untreated controls over 28 days, the CFU increased from 5 to 7 log CFU/g. GSE alone provided no inhibition of L. monocytogenes cell growth at this temperature. Nisin alone gave an initial 2-log reduction but by day 28 the count increased to 6.3 log CFU/g. Conversely, the nisin-GSE combination treatment dip gave the same initial 2-log reduction seen for nisin alone but by day 21 the reduction in surviving cell numbers was below the minimum level of detection, 100 CFU/g, and this inhibition persisted through day 28 (Table 2).

At 10°C, the untreated controls surprisingly increased
only by 2 log by day 28, reaching the same level of 7 log CFU/g as seen at the 4°C incubation. In parallel with the results obtained at 4°C, the single treatments of GSE or nisin alone gave essentially no inhibition of L. monocytogenes growth as compared to the controls. However, the combination treatment of GSE with nisin gave an initial reduction of about 1.5 to 2 log and survivor counts by day 21 were below the minimum level of detection of 100 CFU/g (Table 3).

The predominant phenolics reported by Rababah et al. (21) in the mega natural GSE were epicatechin (1,158.5 mg/100 g), catechin (887.4 mg/100 g), gentisic acid (472.8 mg/100 g), and syringic acid (253.4 mg/100 g). Antimicrobial activities of these individual phenolic constituents have already been demonstrated (2, 8). Epicatechin and catechin have been found to have strong antimicrobial activities against the selected pathogens, Porphyromonas gingivalis and Prevotella intermedia (11). Gentisic and syringic acids were also found to be effective against several gram-positive and gram-negative bacteria and molds (3). It is possible that the phenolic constituents in GSE inhibited L. monocytogenes by binding the cell wall components (cell envelope proteins) and extracellular enzymes (5, 24), or disturbing the cytoplasmic membrane and causing leakage of cellular compounds and interfering with the proton motive force (8, 14).

Several studies of nisin in meat systems indicated that nisin alone was ineffective as compared to other bacteriocins such as pediocin against L. monocytogenes (18). Also Janes et al. (12) demonstrated that the nisin alone in corn zein coatings did not have significant inhibitory effects against L. monocytogenes on cooked chicken breast after 24 days storage at 4 and 8°C. However, a combination dip treatment of nisin (6,400 IU) with GSE (1%) exerted greater inhibitory effects than either compound alone against L. monocytogenes on full-fat frankfurters at 4 or 10°C. This combination can improve the antimicrobial efficacy and overcome the practical problems associated with the use of nisin alone against L. monocytogenes in refrigerated RTE meat and poultry storage.

ACKNOWLEDGMENT

The financial support for this research study by the Food Safety Consortium is greatly appreciated.

REFERENCES