Research Note

Control of *Listeria monocytogenes* with Combined Antimicrobials on Beef Franks Stored at 4°C

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ABSTRACT

Contamination of ready-to-eat meat products such as beef franks with *Listeria monocytogenes* has become a major concern for the meat processing industry and an important food safety issue. The objective of this study was to determine the effectiveness of combinations of antimicrobials as aqueous dipping solutions to control *L. monocytogenes* on vacuum-packaged beef franks stored at 4°C for 3 weeks. Commercial beef franks were dipped for 5 min in three antimicrobial solutions: pediocin (6,000 AU), 3% sodium diacetate and 6% sodium lactate combined, and a combination of the three antimicrobials. Samples were then inoculated with 10⁷ CFU/g of either four *L. monocytogenes* strains individually or a cocktail of the four strains, vacuum packaged, and stored at 4°C for 3 weeks. Sampling was carried out at day 0 and after 2 and 3 weeks of storage. Individual strains, as well as the cocktail, exhibited different responses to the antimicrobial treatments. After 2 and 3 weeks of storage at 4°C, pediocin-treated beef franks showed a less than 1-log reduction for all bacterial strains. Samples treated with the sodium diacetate–sodium lactate combination showed about a 1-log reduction after 2 weeks of storage for all strains and between a 1- and 2-log reduction after 3 weeks of storage, depending on the bacterial strain. When the three antimicrobials were combined, reductions ranged between 1 and 1.5 log units and 1.5 to 2.5 log units after 2 and 3 weeks of storage, respectively, at 4°C. These results indicate that the use of combined antimicrobial solutions for dipping treatments is more effective at inhibiting *L. monocytogenes* than treatments using antimicrobials such as pediocin separately.

*L. monocytogenes*, a widely recognized foodborne pathogen, has reemerged in the United States following a listeriosis outbreak between 1998 and 1999 that caused 21 deaths and more than 100 illnesses in 14 states from the consumption of postprocess-contaminated hot dogs and deli meats (2). The pathogen is widespread in the environment and has the ability to colonize meat processing plants (14). Hygienic and sanitation practices applied in meat processing plants are often insufficient to prevent contamination of processed meat products (4). Contaminating *L. monocytogenes* can be resistant to many food preservation methods (9) and can increase to high numbers during refrigerated storage and under low oxygen tension (4, 5, 7, 14). Thus, effective hurdle technologies to inhibit growth of the pathogen are needed.

Research undertaken in recent years has indicated that bactericidal compounds such as organic acids and bacteriocins can control *L. monocytogenes* in meat products (13, 16, 19). A 5% sodium diacetate dipping solution inhibited pathogen growth throughout storage (120 days) on sliced pork bologna, and 5% lactic acid prevented growth for up to 90 days when the product was stored at 4°C (15). Furthermore, combinations of lactate and diacetate have been shown to exhibit greater antilisterial activity than when either compound is used alone (12, 16). On the other hand, several studies have shown that bacteriocins such as pediocin are bactericidal to *L. monocytogenes* in meat products. Chen et al. (3) have shown that 3,000 and 6,000 AU of pediocin reduced the population of *L. monocytogenes* by 1.5 and 2.0 log units, respectively, on frankfurters stored at 4°C for up to 12 weeks. However, the combined effects of these antimicrobials (pediocin, sodium lactate, and sodium diacetate) used as dipping solutions to inhibit *L. monocytogenes* in ready-to-eat (RTE) meat products have not been reported. In addition to the potential for providing increased antilisterial activity, combinations of antimicrobials might lessen negative effects on the sensory quality of food products that might otherwise occur if the antimicrobials were used singly in the high concentrations required to ensure safety. The purpose of this study was to evaluate the survival of *L. monocytogenes* on beef franks dipped in antimicrobial solutions and stored at 4°C for 3 weeks.

**MATERIALS AND METHODS**

Bacterial strains, food products, and antimicrobials. Four *L. monocytogenes* strains were used: LM 108M (serotype 1/2b, hard salami isolate), LM 101M (serotype 4b, beef and pork sausage isolate), H7776 (serotype 4b, frankfurter isolate), and F6854 (serotype 1/2a, frankfurter isolate). Stock cultures were maintained in cryovials (Pro-Lab Diagnostics, Austin, Tex.) at −80°C and activated by transferring 100 μl into tryptic soy broth (TSB) with yeast extract (TSBYE; Difco, Becton Dickinson, Sparks, Md.) and incubating at 37°C for 24 h. Strains were subcultured twice in...
TABLE 1. MICs of sodium diacetate (SD) and sodium lactate (SL) in TSBYE and of pediocin (P) in TSAYE (0.75% agar) for four L. monocytogenes strains and the four-strain cocktail

<table>
<thead>
<tr>
<th>Strain</th>
<th>SD (%)</th>
<th>SL (%)</th>
<th>P (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM 108M</td>
<td>0.5</td>
<td>&gt;6</td>
<td>1,200</td>
</tr>
<tr>
<td>LM 101M</td>
<td>0.5</td>
<td>&gt;6</td>
<td>30,000</td>
</tr>
<tr>
<td>H7776</td>
<td>0.4</td>
<td>&gt;6</td>
<td>18,800</td>
</tr>
<tr>
<td>F6854</td>
<td>0.4</td>
<td>&gt;6</td>
<td>3,600</td>
</tr>
<tr>
<td>Cocktail</td>
<td>0.5</td>
<td>&gt;6</td>
<td>1,600</td>
</tr>
</tbody>
</table>

Statistical analysis. Statistical analysis was performed by the Design-Expert (Design of Experiment) software (version 6, Stat-Ease Inc., Minneapolis, Minn.). Analysis of variance was performed to determine statistically significant differences \((P \leq 0.05)\) of mean values between treated and control samples and between treatments at each sampling time.

RESULTS

MIC assay. The MICs for SD, SL, and pediocin are recorded in Table 1. The MICs for SD were 0.4% for strains H7776 and F6854 and 0.5% for strains LM 108M, LM 101M, and the four-strain cocktail. The MIC for SL was not reached because all strains showed growth at 6%, which is the maximum concentration organoleptically acceptable for dipping treatments. The MICs for pediocin were 1,200 AU (0.6%) for LM 108M, 1,600 AU (0.8%) for the cocktail, 3,600 AU (1.8%) for F6854, 18,800 AU (9.4%) for H7776, and 30,000 AU (15%) for LM 101M.

Inhibition of L. monocytogenes by antimicrobials on beef franks. For this study, beef franks were dipped in sterile deionized water (control); in a solution containing 3% pediocin; in a solution containing 3% SD and 6% SL; and in a solution containing 3% SD, 6% SL, and 3% pediocin. The concentrations chose were the maximum levels that were organoleptically acceptable as determined by previous research \((3, 6)\). The SD-SL solution was prepared in deionized water and autoclaved. Pediocin solution was aseptically prepared in sterile, deionized water.

Beef franks were immersed in the antimicrobial test solution for 5 min and then allowed to dry for 20 min. The L. monocytogenes overnight culture (1 ml) was then surface inoculated onto the product, spread evenly, and left to dry for 5 min. After treatment, each beef frank was placed into a vacuum bag (FoodSaver, Tilia, San Francisco, Calif.), vacuum sealed (FoodSaver BagVac, Tilia), and stored at 4°C for 3 weeks. At each sampling time, 25 g of the test product was transferred into a sterile stomacher bag (Whirl-Pak, Nasco, New Haven, Conn.), 225 ml of buffered peptone water (Difco) was added, and the contents were mixed for 1 min at 230 rpm in a Stomacher 400 (Seward Limited, London, UK). For each sample, appropriate serial dilutions were made in buffered peptone water and plated in duplicate on brain heart infusion (BHI; Difco) and on modified Oxford formulation (MOX; Difco) agar, which were used as nonselective and selective media, respectively. Colonies were enumerated after incubation at 37°C for 24 to 48 h. Three replications of all experiments were done.

To confirm the uniformity of bacterial distribution with the above-mentioned surface inoculation method, beef franks were surface inoculated, cut into equal 20-g halves, and sampled for enumeration as mentioned before. Colonies were enumerated after incubation at 37°C for 24 to 48 h. The pH of the beef franks was measured with a surface electrode (model 520A, Orion, Beverly, Mass.).
FIGURE 1. Survival of L. monocytogenes LM 108M on beef franks surface treated with 3% pediocin; a combination of 3% SD and 6% SL (SD-SL); or a combination of 3% SD, 6% SL, and 3% pediocin (SD-SL-pediocin) and stored at 4°C for 3 weeks. Values plotted at each time point are an average of three replications. Error bars represent standard deviation from the mean.

After 2 weeks, pediocin-treated beef franks showed no reduction, whereas samples treated with SD-SL and SD-SL-pediocin showed about a 1.0-log reduction in their populations. After 3 weeks, a 1.4-log reduction was observed in samples treated with SD-SL-pediocin. No difference was observed between treatments used either alone or in combination after 3 weeks of storage at 4°C.

L. monocytogenes strain H7776 was the most sensitive organism to the 3% pediocin treatment. After 2 and 3 weeks of storage, a 1.0- and 1.4-log reduction, respectively, was observed in the viable population (Fig. 3). For this strain, the SD-SL treatment rendered an effect similar to the pediocin treatment after both 2 and 3 weeks of storage. The largest decrease (1.8 log units) was observed when beef franks were treated with the combined antimicrobial solution.

L. monocytogenes F6854 showed only a 0.5-log reduction when beef franks were dipped in 3% pediocin and stored for 2 and 3 weeks at 4°C (Fig. 4). For this strain, a 1.3- and 1.4-log reduction was observed for the SD-SL and the combined antimicrobial treatments, respectively, after both 2 and 3 weeks of storage.

The four-strain cocktail showed about a 0.5-log reduction when beef franks were treated with pediocin and stored for 2 weeks at 4°C (Fig. 5). Both the SD-SL and the SD-SL-pediocin treatments reduced the population by about 1.0 log units after 2 weeks of storage. The cocktail showed the greatest reduction of all tested organisms for both the SD-SL dipping treatment (2.0 log units) and the combined antimicrobial treatment (2.5 log units) when beef franks were stored at 4°C for 3 weeks.

DISCUSSION

Results from our culture studies showed that SL at a concentration of up to 6% did not prevent L. monocytogenes growth (Table 1). These results are consistent with find-
ings by Shelef and Yang (18), who observed that SL at a concentration of 7.8% delayed but did not suppress growth of three *L. monocytogenes* strains in TSB. In another study, Blom et al. (1) observed that 3.5% SL inhibited growth of *L. monocytogenes* in liquid media at pH 5.5, but only a small degree of inhibition was observed when the same concentration of SL was tested at pH 6.3. MICs for SD in this study varied from 0.4 to 0.5% among the *L. monocytogenes* strains tested. Similar results have been observed by Shelef and Addala (17), who found that 0.5% SD inhibited growth of *L. monocytogenes* strains Brie-1, DA3, and LCDC in BHI broth.

When determining the MIC for pediocin, our results varied from 1,200 AU (0.6%) to 30,000 AU (15%) among the tested *L. monocytogenes* strains. Similarly, Szabo and Cahill (19) observed MICs varying from 160 AU (0.08%) to 1,200 AU (0.6%) among 21 *L. monocytogenes* strains in TSB with 0.5% No. 1 agar when using ALTA 2341 as the source of pediocin. Jagannath et al. (8) observed a MIC of 800 AU/ml for *L. monocytogenes* Scott A in BHI broth when using pediocin K7 obtained from *Pediococcus acidilactici* CFRK 7. In another study, Nielsen et al. (10) recorded 460 AU/ml as the MIC for *L. monocytogenes* Scott A in TSB with 0.75% agar when pediocin was produced by *P. acidilactici* obtained from a Lactagel 110 (Microlife Technics, Sarasota, Fla.) commercial culture. Results from the different studies suggest that pediocin obtained from different sources could have a varied antilisterial effect and that different *L. monocytogenes* strains have different sensitivity to the bacteriocin. Also, the MICs vary depending on the suspending medium used in the determination.

Results from the beef frank studies showed that the use of a combination of 3% SD, 6% SL, and 3% pediocin as a dipping solution was slightly more effective at reducing *L. monocytogenes* than the SD-SL treatment for some strains and more effective than the pediocin treatment alone for all strains. When comparing the SD-SL treatment with the pediocin treatment, the latter was found to be less effective in most cases. In all cases, the reduction of the *L. monocytogenes* population for the combined antimicrobial

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**FIGURE 3.** Survival of *L. monocytogenes* H7776 on beef franks surface treated with 3% pediocin; a combination of 3% SD and 6% SL (SD-SL); or a combination of 3% SD, 6% SL, and 3% pediocin (SD-SL-pediocin) and stored at 4°C for 3 weeks. Values plotted at each time point are an average of three replications. Error bars represent standard deviation from the mean.

**FIGURE 4.** Survival of *L. monocytogenes* F6854 on beef franks surface treated with 3% pediocin; a combination of 3% SD and 6% SL (SD-SL); or a combination of 3% SD, 6% SL, and 3% pediocin (SD-SL-pediocin) and stored at 4°C for 3 weeks. Values plotted at each time point are an average of three replications. Error bars represent standard deviation from the mean.
FIGURE 5. Survival of L. monocytogenes four-strain cocktail on beef franks surface treated with 3% pediocin; a combination of 3% SD and 6% SL (SD-SL); or a combination of 3% SD, 6% SL, and 3% pediocin (SD-SL-pediocin) and stored at 4°C for 3 weeks. Values plotted at each time point are an average of three replications. Error bars represent standard deviation from the mean.

Treatment was statistically significant ($P \leq 0.05$) compared with control untreated beef franks after both 2 and 3 weeks of storage at 4°C. Glass et al. (6) observed that L. monocytogenes populations in winers stored for 45 days at 4.5°C were 2.0 log CFU per package lower when the product was immersed for 2 min in a solution containing 6% SL and 3% SD compared with the control samples. However, little or no reduction was observed when winers were treated separately with each antimicrobial. In another study, Chen et al. (3) reported immediate reductions of 1.0 log CFU/g in L. monocytogenes populations when frankfurters were sprayed with ALTA 2341 solution at concentrations of 3,000 and 6,000 AU. In this same study, a 1.5- and 2.0-log CFU/g reduction was observed for the 3,000 and 6,000 AU levels, respectively, when the product was stored at 4°C for up to 12 weeks. Schlyter et al. (16) observed that at refrigeration temperature, turkey slurries containing lactate alone at a concentration of 2.5% supported growth of L. monocytogenes, whereas slurries containing lactate with 0.1, 0.3, or 0.5% diacetate were listericidal. Schlyter et al. (16) also reported that 0.5% diacetate alone displayed greater antilisterial activity (1.0-log CFU/ml reduction) than 5,000 AU/ml pediocin alone (4.0-log CFU/ml increase) in turkey slurries at 4 and 25°C. However, when pediocin and diacetate were combined, a 2.0- and 1.5-log CFU/ml reduction was observed for slurries stored for 42 days at 4°C and 7 days at 25°C, respectively.

Strain LM 108M was the most sensitive to the pediocin treatment in the MIC studies on TSAYE, whereas strain H7776 was the most sensitive to pediocin in beef franks. Strain LM 101M was the most resistant to pediocin in the MIC studies and on beef franks. LM 101M was also the most resistant to the SD-SL and the SD-SL-pediocin treatments in beef franks. Overall, the strains did not show a consistent pattern in the MIC studies in TSBYE and TSAYE (0.75% agar) when compared with the beef frank studies. Variations in resistance could be caused by differences in each strain’s ability to adhere to the beef frank surface and by enhanced protection against the antimicrobials given by the protein and fat constituents of the meat product.

From the results of this study, it is clear that L. monocytogenes survived on the beef franks (approximately 10⁷ CFU/g) at 4°C for 3 weeks. If postprocessing contamination of L. monocytogenes occurs, this would be a major safety concern, which emphasizes the need for antimicrobial interventions and measures to control growth of the pathogen. From our results, it is evident that the effect of natural antimicrobials such as pediocin was significantly enhanced when used in combination with other additives such as SD-SL. Thus, the use of these secondary barriers in conjunction with adequate manufacturing and sanitary practices can enhance the safety of RTE meat products.

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REFERENCES


