

# COMBINING ANTIMICROBIALS AND HYDRODYNAMIC PRESSURE PROCESSING FOR CONTROL OF *LISTERIA MONOCYTOGENES* IN FRANKFURTERS\*

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## ABSTRACT

*The safety of ready-to-eat meat products such as frankfurters can be enhanced by antimicrobials and postpackaging treatment to control the growth of *Listeria monocytogenes*. We evaluated the effectiveness of dipping frankfurters in sodium lactate (SL), sodium diacetate (SD) and nisin (N) solutions, followed by hydrodynamic pressure processing (HDP), to control *L. monocytogenes* during storage at 4C for 28 days. Frankfurters were surface inoculated with a five-strain mixture of *L. monocytogenes* after dipping in (1) control (sterile water), (2) 10% SL, (3) 5% SD, (4) SL + SD, (5) N (5000 IU/mL), (6) N + SL, (7) N + SD and (8) N + SL + SD for 5 min. The frankfurters were vacuum packed and were either treated with or without hydrodynamic pressure. The frankfurters were periodically analyzed for populations of *L. monocytogenes* on modified Oxford agar, aerobic cell populations on tryptic soy agar with 0.6% yeast extract and pH on days 0, 7, 14 and 28. HDP treatment significantly reduced *L. monocytogenes* populations in frankfurters. Dipping treatments containing N showed greater *L. monocytogenes* reduction than that with SL and/or SD treatment; however, the difference was not significant. The combination of N and HDP treatment resulted in more than 2 log cfu/g reduction in *L. monocytogenes* populations after 28-days storage. A synergistic effect between N and HDP was observed for inhibition of *L. monocytogenes*. The reduction in aerobic cell populations during storage*

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*of antimicrobials and HDP-treated frankfurters was similar to that of L. monocytogenes. These results indicate that using N as a dipping treatment followed by HDP treatment is more effective for inhibition of the pathogen during storage at 4C.*

## PRACTICAL APPLICATIONS

Hydrodynamic pressure processing, an innovative post-harvest technology for meat tenderization, uses a high-energy explosive to generate a supersonic shock wave in a liquid medium. HDP treatment, primarily studied for tenderness improvement in muscle food products, has been effective in reducing bacterial populations in meats. Nisin has greater antilisterial activity than sodium lactate or sodium diacetate. Dipping frankfurters in nisin followed by the hydrodynamic pressure treatment could be used to reduce 2 log *L. monocytogenes* populations in frankfurters during storage at 4C.

## INTRODUCTION

*Listeria monocytogenes* is a foodborne pathogen of concern in ready-to-eat (RTE) meat products. It is ubiquitous in nature, is difficult to completely remove from the processing environments and is known for its ability to survive in refrigerated environments. Of the 38.6 million cases of foodborne illness in the U.S.A. caused by known pathogens annually, *L. monocytogenes* caused an estimated 2518 cases resulting in 2322 hospitalizations and 504 deaths (Mead *et al.* 1999). While most healthy people are less susceptible to foodborne listeriosis, populations that are particularly susceptible to infection include pregnant women, the immunocompromised, the young and the elderly (Jay 1996).

In response to consumers' demands for convenience foods, there has been a growing market for RTE processed meat products. RTE meat products processing involves slicing and packaging operations after terminal heat treatment that may represent an opportunity for postprocessing contamination (Lopez-Caballero *et al.* 2002). The U.S.A. has a "zero-tolerance" policy on *L. monocytogenes* in RTE meat products, meaning the organism must be absent in 25-g samples tested; otherwise, the product is considered adulterated (Jay 1996). Some notable outbreaks of listeriosis in RTE meat products include a 1998 multistate outbreak involving beef franks that resulted in 15 deaths, and another in 2002 in the northeastern U.S.A. involving sliceable turkey deli meat (CDC 1998, 2002).

Bactericidal effects of organic acids and bacteriocins in various foods have been documented. Organic acids such as sodium lactate (SL) and sodium diacetate (SD) have been used as antimicrobials in product formulations or applied as a surface treatment on finished products. Beef franks inoculated with *L. monocytogenes* and dipped into 6% SL, 3% SD or a combination of the two showed a 1–2 log<sub>10</sub> cfu/g reduction after 2 weeks of refrigerated storage (Uhart *et al.* 2004). SL (1.32–3.40%) and SD (0.10 and 0.25 %) in wiener or bratwurst product formulation inhibited *L. monocytogenes* growth after 4–12 weeks of refrigerated storage (Glass *et al.* 2002). In addition to organic acids, bacteriocins such as nisin (N) or pediocin can also be applied to products. Bacteriocins are effective against gram-positive bacteria, but are ineffective against gram negatives (Zhang and Mustapha 1999; Gill and Holley 2000). Populations of *L. monocytogenes* inoculated on beef cubes treated with N and then vacuum packaged were reduced by 2.01 log cfu/cm<sup>2</sup> after storage at 4C for up to 30 days (Zhang and Mustapha 1999). Pediocin, alone or in combination with organic acids, reduced populations of *L. monocytogenes* on beef franks stored at 4C for up to 4 weeks (Uhart *et al.* 2004).

Research has been conducted to determine the effectiveness of different processing technologies as interventions to reduce or eliminate *L. monocytogenes* and other pathogens in various products. High pressure processing (HPP) and irradiation have been studied in combination with antimicrobials to reduce microorganisms in meats. HPP, with pressures in the range of 300–700 MPa, can significantly reduce bacterial populations while maintaining the sensory quality of RTE meats (Hayman *et al.* 2004). Ionizing radiation eliminates *L. monocytogenes* in RTE meat products (Sommers *et al.* 2002; Foong *et al.* 2004). Another potential food safety intervention is hydrodynamic pressure processing (HDP). This process uses a high-energy explosive to generate supersonic shockwaves in a liquid medium and was initially developed for improving meat tenderness (Solomon *et al.* 1997). The shock waves generated in the HDP process reduced spoilage bacteria on the surface of beef stew pieces (Williams-Campbell and Solomon 2002), on pork stew pieces and in ground beef (Williams-Campbell and Solomon 2000), and on intact beef muscle (Schilling *et al.* 2003). HDP treatment resulted in marginal reduction of *Salmonella* populations in minced chicken (Patel *et al.* 2006), whereas significant reduction of *L. monocytogenes* populations on beef surfaces was reported (Patel and Solomon 2005). HDP may be applied as an in-package lethal treatment for packaged RTE meats that may have been contaminated during slicing, comminuting and/or packaging. Currently, no information is available on combining antimicrobials with HDP as an intervention processing to reduce *L. monocytogenes* in RTE meats and its subsequent bactericidal effect during storage of such products. The objective of this study was to investigate the effect of HDP, in conjunction with SL, SD and N alone or in

combination, on *L. monocytogenes* populations on frankfurters. The effect of HDP and antimicrobials on control of *L. monocytogenes* during refrigerated storage was also investigated.

## MATERIALS AND METHODS

### Bacterial Strains

Five strains of *L. monocytogenes* were used in this study. Strain Scott A was obtained from the culture collection at our laboratory. Four strains isolated from meats were also used: LM 101M (serotype 4b, beef and pork sausage isolate), LM 108M (serotype 1/2b, hard salami isolate), H7776 (serotype 4b, frankfurter isolate) and F6854 (serotype 1/2a, frankfurter isolate). Meat isolates were graciously provided by Dr. Ravishankar (National Center for Food Safety and Technology, Summit-Argo, IL). Cultures were maintained in sterile 2.0-mL cryovials (Nalgene, Rochester, NY) containing tryptic soy broth (TSB) with yeast extract (TSBYE; Acumedia, Lansing, MI) and 20% glycerol (Fisher Scientific, Suwanee, GA), and then stored at  $-80^{\circ}\text{C}$ . The cultures were individually activated by transferring a single loopful of culture to 10-mL sterile TSBYE and incubated at  $35^{\circ}\text{C}$  for 24 h. After two successive transfers, all five strains were mixed in equal proportions into a sterile tube and were vortexed to prepare the cocktail.

### Antimicrobials and Frankfurters

Three antimicrobials were used in this study. N (2.5%, balance sodium chloride and denatured milk solids) and SD were obtained from Sigma-Aldrich (Allentown, PA). SL (Ultrapure SL-75, 60% aqueous.) was obtained from Trumark, Inc. (Linden, NJ). N stock solutions were prepared by dissolving 0.5-g N in 100-mL 0.02 N HCl, adjusting the pH to 6.0 and filter sterilizing in a 150-mL vacuum filter unit containing 0.45- $\mu\text{m}$  polyvinylidene fluoride membrane filter (Millipore, Billerica, MA). Stock solutions (10,000 IU/mL) were stored at  $4^{\circ}\text{C}$  until ready for use. Commercially prepared beef franks were purchased from local grocery stores.

### Minimum Inhibitory Concentration (MIC) Assay

The procedure for the MIC assay was modeled after the experiments conducted by Uhart *et al.* (2004). The MIC assay was performed to determine the MIC of each of the antimicrobials on individual *L. monocytogenes* strains. Solutions were prepared with different concentrations of SL (0–10%), SD (0–0.8%) and N (0–1000 IU/mL) in 10-mL aliquots of TSBYE. Test solutions

were inoculated with 0.1 mL ( $\sim 7 \log_{10}$  cfu/mL) of each *L. monocytogenes* isolate. Tubes were incubated at 35C for 24 h. Lack of turbidity after incubation indicated inhibition. The MIC was recorded as the lowest concentration that resulted in no visible turbidity after incubation.

### Sample Preparation

Thirty-two beef franks were divided into two groups designated for no-HDP and HDP treatment. There were eight treatments, with two franks assigned to each treatment. The beef franks were dipped into treatment solutions contained in sterile ninth-size polycarbonate food pans (Ace Mart Restaurant Supply, San Antonio, TX) for 5 min (25C). Treatments consisted of the following: (1) sterile distilled water (control), (2) 10% SL, (3) 5% SD, (4) 10% SL + 5% SD, (5) N (5000 IU/mL), (6) 5000-IU/mL N + 10% SL, (7) 5000-IU/mL N + 5% SD and (8) 5000-IU/mL N + 10% SL + 5% SD. After 5-min dipping treatment, the beef franks were then air dried on sterile racks for 10 min. The franks were then dipped into sterile 1/6th stainless steel steam table pans (Ace Mart Restaurant Supply) containing  $7 \log_{10}$  cfu/mL of the *L. monocytogenes* cocktail inoculum. The franks were dipped in the suspension of *L. monocytogenes* for 5 min at 25C to allow bacterial attachment to the surface, and were then air dried on sterile racks for 10 min.

After drying, the beef franks were separated into their respective treatments then placed into 15.2 × 25.4-cm (6 × 10 in.) nylon vacuum pouches (3 mil standard barrier; Cryovac Sealed Air Corp., Duncan, SC) and vacuum packaged (model LV 10 G; Hollymatic Corp., Countryside, IL). Packages designated for HDP treatment were further vacuum packaged in 35.5 × 66.0-cm boneguard bags (Cryovac Sealed Air) and heat shrunk in 88C water for 1 s to remove any air pockets in the bags. Following treatments, the vacuumed packages were stored at 4C for 28 days.

### HDP

HDP was conducted indoors in a 54-L stainless steel shockwave container. A heat-shrunk boneguard bag containing treated and inoculated meat was secured at the bottom of the shockwave container, and the container was filled with water. A 100-g binary explosive (rectangle shaped) was immersed in the 4C water 30.5 cm above the meat surface. The steel lid on the container was secured and locked, and the explosive was detonated.

### Microbiological Analyses

Surviving populations of *L. monocytogenes* following antimicrobials and HDP treatments as well as 7, 14 and 28-days storage period at 4C were

evaluated. Because of the lack of HDP-processed sample availability, extended storage study was not feasible. The packages were aseptically opened, and the frankfurters were placed on sterile trays. The rounded edges of the franks were removed with a sterile knife, and an approximately 2.54-cm sample (~20 g) was removed for microbial analysis. The sample was placed in a sterile stomacher bag with a filter (Microbiology International, Frederick, MD) and was serially diluted with sterile 0.1% peptone water using an automatic diluter (Dilumat 3, Microbiology International). The sample was pummeled for 2 min using a stomacher (BagMixer; Interscience, St. Nom, France). Appropriately diluted samples were spiral plated (100  $\mu$ l; WASP2, Microbiology International) onto duplicate plates of tryptic soy agar with 0.6% yeast extract (TSAYE, Acumedia) and modified Oxford agar (MOX, BD Sparks, MD). The plates were incubated at 35C for 48 h, and were then counted using the ProtoCOL automated colony counter (Microbiology International).

### **pH Determination**

The procedure described by Peachey *et al.* (2002) was used for pH measurement. Briefly, a 2–2.5-g beef frank sample was placed into a sterile 50-mL centrifuge tube with a conical bottom (VWR International, West Chester, PA). Ten milliliters of iodoacetate solution (5-mM iodoacetate in 150-mM KCl; Sigma-Aldrich) was added to the sample. The sample was then homogenized using a Polytron homogenizer with a 12-mm foam-reducing generator (Brinkmann Instruments, Westbury, NY). The pH was measured using a Denver Instrument model 250 pH meter (Denver Instrument, Denver, CO).

### **Data Analysis**

All experiments were performed three times. The mean values of two plates from each of three replicates were converted to  $\log_{10}$  cfu/g. The data were analyzed by three-way analysis of variance using a 'Proc Mixed' statement (SAS 8.2; SAS Institute Inc., Cary, NC) for effects of treatment, antimicrobials, storage time and interaction. The Sidak adjustment was utilized to protect against inflation of the Type 1 error. The effects of antimicrobials, storage time and interaction on pH changes were analyzed as previously described. In all cases, a statistical significance level of  $P < 0.05$  was used.

## **RESULTS**

### **MIC Assay**

The MIC for SL could not be reached as all strains showed weak to moderate growth at 6%. The MICs for SD were 0.4% for all strains. The

inhibitory activity of N varied with *Listeria* strain. The MIC for strains for Scott A, 108M and H7776 was 300 IU/mL strain, and the MIC for strains 101M and F6854 was 400 IU/mL.

### Effect of Antimicrobials on pH

The initial pH of frankfurters was 5.68 (Table 1). Addition of SL or N did not affect the meat pH, but SD reduced it to 5.55. The combination of SD with SL or N also reduced the initial pH to 5.52 and 5.59, respectively. The data suggested that dipping frankfurters in acids or combinations of acids and N did not significantly change the pH of frankfurters. As expected, HDP treatment did not influence the pH of the meat either. A small pH increase of 0.1–0.3 pH units was observed in both antimicrobial and HDP-treated samples during refrigerated storage. Sidak-adjusted mean comparisons for each antimicrobial and treatment interactions revealed no significant increase in pH during storage of frankfurters with one exception. The pH of the SL + SD + N and HDP-treated samples at day 28 (5.84) was significantly higher than that of the day 0 sample (5.53).

TABLE 1.  
LEAST SQUARE MEANS FOR pH VALUES OF FRANKFURTERS TREATED WITH  
ANTIMICROBIALS FOLLOWED BY HDP, AND STORED AT 4C

Treatment*	Dip solution†	Time (days)			
		0	7	14	28
Control	Water	5.68 (0.03)‡	5.93 (0.06)	5.75 (0.11)	5.95 (0.13)
	SL	5.71 (0.11)	5.79 (0.19)	5.75 (0.14)	5.86 (0.05)
	SD	5.55 (0.10)	5.71 (0.01)	5.60 (0.20)	5.79 (0.05)
	SL + SD	5.52 (0.07)	5.67 (0.13)	5.61 (0.22)	5.73 (0.08)
	N	5.66 (0.06)	5.76 (0.12)	5.74 (0.14)	5.81 (0.10)
	N + SL	5.73 (0.11)	5.79 (0.10)	5.82 (0.18)	5.90 (0.03)
	N + SD	5.59 (1.01)	5.70 (0.08)	5.75 (0.15)	5.77 (0.05)
	All	5.64 (0.09)	5.70 (0.09)	5.76 (0.07)	5.76 (0.08)
HDP	Water	5.69 (0.11)	5.81 (0.19)	5.86 (0.06)	5.84 (0.05)
	SL	5.71 (0.03)	5.69 (0.26)	5.90 (0.16)	5.88 (0.03)
	SD	5.60 (0.12)	5.68 (0.14)	5.72 (0.23)	5.82 (0.10)
	SL + SD	5.66 (0.03)	5.67 (0.19)	5.80 (0.10)	5.80 (0.10)
	N	5.77 (0.07)	5.71 (0.20)	5.82 (0.05)	5.85 (0.07)
	N + SL	5.77 (0.05)	5.76 (0.20)	5.83 (0.09)	5.87 (0.03)
	N + SD	5.62 (0.09)	5.67 (0.15)	5.75 (0.08)	5.80 (0.05)
	All	5.53 (0.07)	5.71 (0.12)	5.70 (0.17)	5.84 (0.04)

\* Control, not treated with HDP.

† Water, control; SL, sodium lactate; SD, sodium diacetate; N, Nisin; all, SL, SD and N combined.

‡ Standard deviation.

HDP, hydrodynamic pressure processing.

### *L. monocytogenes* Inhibition by Antimicrobials

*Listeria monocytogenes* was not detected on MOX plates, and very few ( $<1 \log_{10}$  cfu/g) aerobic populations were detected on TSAYE plates from uninoculated beef frankfurters not treated with antimicrobials and HDP. The behavior of *L. monocytogenes* on frankfurters after treatment with antimicrobials and HDP is presented in Fig. 1A. The initial *L. monocytogenes* population in untreated beef franks (inoculated controls) was  $5.57 \log_{10}$  cfu/g. The HDP treatment significantly reduced *L. monocytogenes* populations to  $4.61 \log_{10}$  cfu/g. Dipping in SL, SD or their combinations resulted in marginal reduction ( $<0.30 \log_{10}$  cfu/g) of *L. monocytogenes*. While *L. monocytogenes* reduction was greater on frankfurters treated with N alone or in combination with acids (ca.  $0.55 \log_{10}$  cfu/g), the effect was not significant. Overall, the bactericidal effect of HDP treatment was greater than the effects of SL, SD or N. *Listeria monocytogenes* populations on beef franks treated with SL, SD or the combination of SL and SD and HDP were not significantly reduced when compared with counts on beef franks that were only treated with HDP. However, the combination of N and HDP processing reduced *L. monocytogenes* populations to  $3.9 \log_{10}$  cfu/g from initial *L. monocytogenes* populations of  $5.57 \log_{10}$  cfu/g. Significant *L. monocytogenes* reductions were achieved on

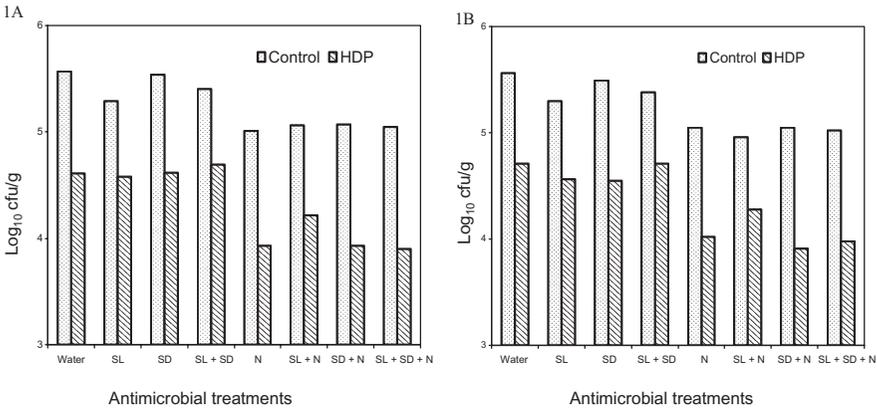


FIG. 1. SURVIVAL OF *LISTERIA MONOCYTOGENES* AND AEROBIC MICROORGANISMS ON THE SURFACE OF FRANKFURTERS TREATED WITH ANTIMICROBIALS AND POSTPACKAGING HYDRODYNAMIC PRESSURE TREATMENT

(A) *Listeria monocytogenes* on modified Oxford agar and (B) aerobic plate counts on tryptic soy agar with 0.6% yeast extract. [Dotted bar] control, nontreated with hydrodynamic pressure; [Hatched bar] HDP, hydrodynamic pressure processing. Ten percent sodium lactate (SL), 5% sodium diacetate (SD) and 400-IU/mL nisin (N) concentrations were used for dipping. Standard deviation varied from 0.04 to 0.38.

frankfurters when N, N + SD or N + SD + SL were used in combination with HDP compared with franks that were only HDP treated.

The data for aerobic bacterial populations in Fig. 1B (TSAYE) were similar to the results for *L. monocytogenes* in Fig. 1A (MOX). Bacterial populations recovered on TSAYE were identical or greater than those recovered on MOX agar. TSAYE medium would not only allow the growth of other microorganisms but would also permit recovery of injured *L. monocytogenes* cells. HDP treatment significantly reduced aerobic populations on frankfurters from 5.57 to 4.71 log<sub>10</sub> cfu/g. Colonies grown on TSAYE were predominantly *L. monocytogenes* resembling uniform appearance of typical *Listeria* colonies. Bacterial recovery on TSAYE and MOX following antimicrobial treatments and HDP followed a similar pattern.

### Effect of Antimicrobials during Storage

The surviving populations of *L. monocytogenes* on treated beef franks during storage at 4C are shown in Fig. 2. In general, *L. monocytogenes* counts were reduced during the 28-day storage period by all antimicrobials and their combinations (Fig. 2A). For each sampling period of 7 and 14 days, *L. monocytogenes* populations detected in frankfurters treated with N or in combination with N were always lower than those from SL or SD-treated frankfurters. The *L. monocytogenes* populations recovered on 28-days stored frankfurters that were treated with N (4.23 log<sub>10</sub> cfu/g), SL + N (4.10 log<sub>10</sub> cfu/g), SD + N (4.32 log<sub>10</sub> cfu/g) and N + SL + SD (4.17 log<sub>10</sub> cfu/g) were lower ( $P > 0.05$ ) than the *L. monocytogenes* populations obtained from control samples (4.70 log<sub>10</sub> cfu/g). While N alone or in combination with organic acids exerted greater antilisterial effect compared with SL or SD alone, the reduction in *L. monocytogenes* populations on treated frankfurters obtained on specific sampling days 7, 14 or 28 was not significant.

Aerobic bacterial populations in antimicrobials treated frankfurters decreased during 4C storage (Fig. 2B). Cell populations recovered on TSAYE were identical to those recovered on MOX agar. Aerobic populations recovered from franks treated with N alone or in combination with organic acids after 28-days storage were between 4.07 and 4.34 log<sub>10</sub> cfu/g, and not significantly lower than counts recovered from SL or SD-treated franks (4.50–4.74 log<sub>10</sub> cfu/g) on day 28. When compared at each sampling period of 7, 14 or 28 days, aerobic cell populations obtained from all antimicrobial-treated frankfurters were similar ( $P > 0.05$ ).

A trend of *L. monocytogenes* reduction on beef franks treated with antimicrobials and HDP was similar to that on beef franks treated with only antimicrobials (Fig. 3A). Combining N with SL or SD before the HDP treatment did not exert additional *L. monocytogenes* reduction on frankfurters as

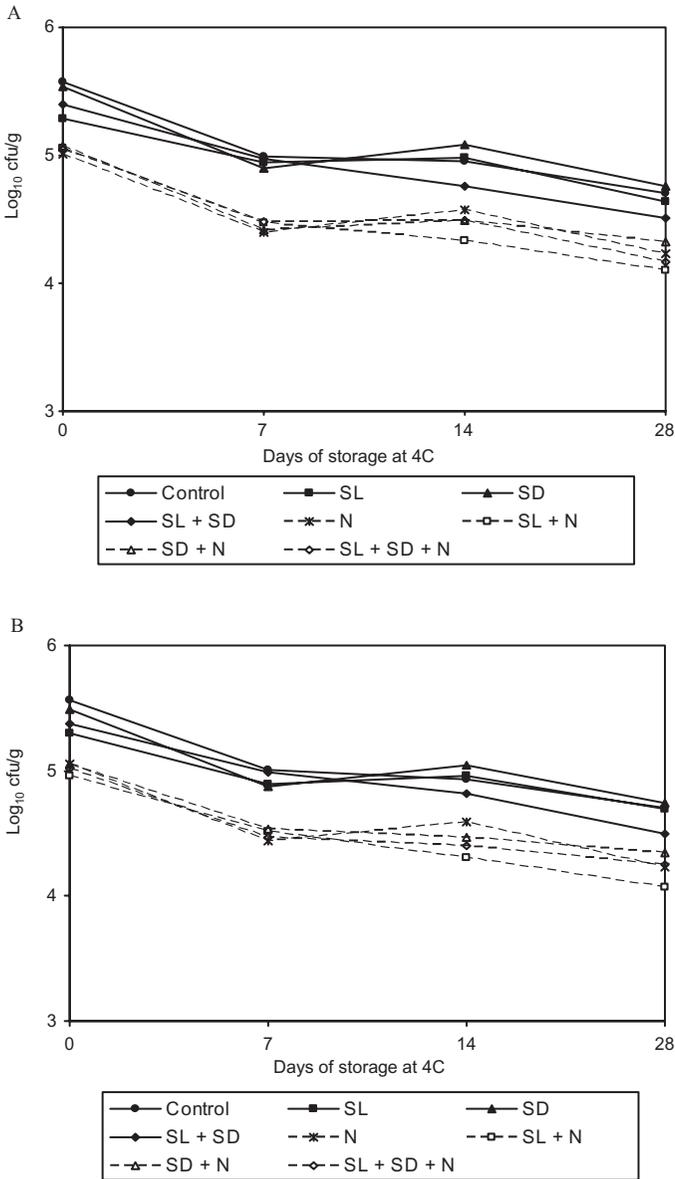


FIG. 2. SURVIVAL AND GROWTH OF *LISTERIA MONOCYTOGENES* AND AEROBIC BACTERIA ON THE SURFACE OF FRANKFURTERS TREATED WITH ANTIMICROBIALS DURING STORAGE AT 4C

(A) *Listeria monocytogenes* on modified Oxford agar and (B) aerobic plate counts on tryptic soy agar with 0.6% yeast extract. Ten percent sodium lactate (SL), 5% sodium diacetate (SD) and 400-IU/mL nisin (N) concentrations were used for dipping. Standard deviation varied from 0.03 to 0.42.

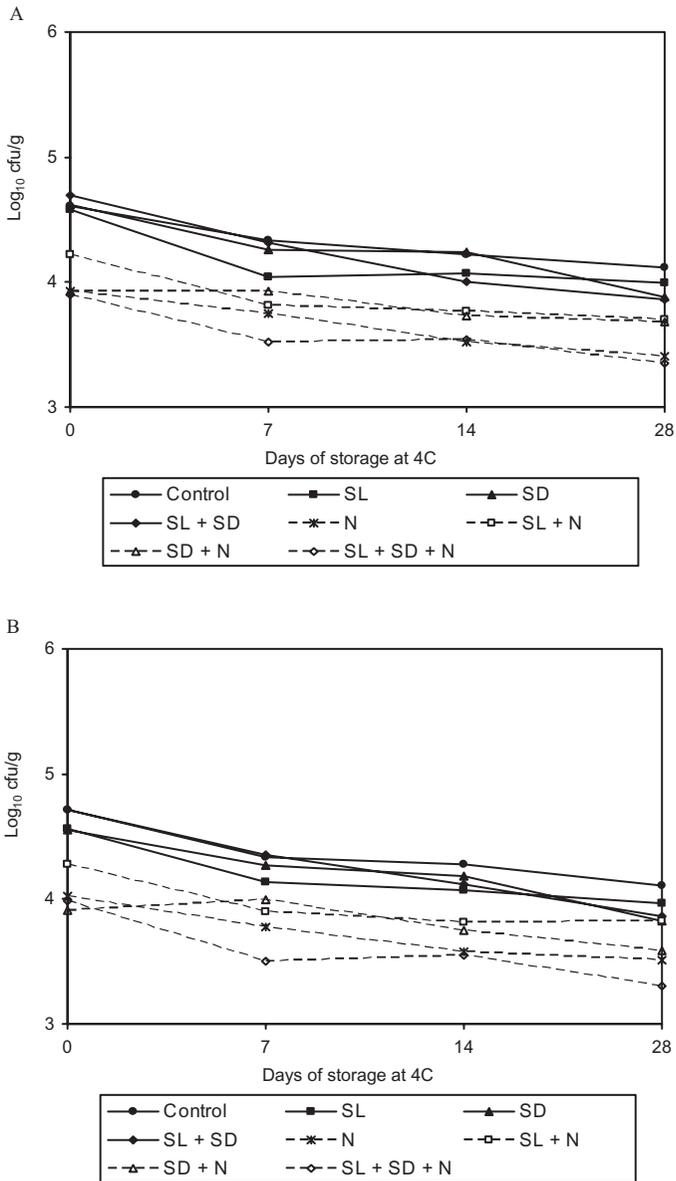


FIG 3. SURVIVAL AND GROWTH OF *LISTERIA MONOCYTOGENES* AND AEROBIC BACTERIA ON THE SURFACE OF FRANKFURTERS TREATED WITH ANTIMICROBIALS AND HYDRODYNAMIC PRESSURE PROCESSING DURING STORAGE AT 4C (A) *Listeria monocytogenes* on modified Oxford agar and (B) aerobic plate counts on tryptic soy agar with 0.6% yeast extract. Ten percent sodium lactate (SL), 5% sodium diacetate (SD) and 400-IU/mL nisin (N) concentrations were used for dipping. Standard deviation varied from 0.04 to 0.38.

evident from surviving *L. monocytogenes* populations of ca. 3.75 log<sub>10</sub> cfu/g after 14 and 28 days compared with N alone after 14 and 28 days (3.52 and 3.41 log<sub>10</sub> cfu/g, respectively) of storage. After storage of 7 and 28 days, *L. monocytogenes* populations recovered in frankfurters treated with N + SL + SD and HDP were significantly lower than the counts detected from the sample with only HDP treatment. The antilisterial effectiveness of N was enhanced by combination with HDP, and there was a significant interaction between HDP and N, suggesting a synergistic effect of these two treatments on *L. monocytogenes*. Aerobic bacterial populations in frankfurters treated with antimicrobials and HDP decreased during the storage period (Fig. 3B). In general, populations recovered on TSAYE were greater than those on MOX agar. N alone or in combination with SL and/or SD showed greater bactericidal effect during storage compared with the effect of SL or SD. No antimicrobial treatment significantly reduced aerobic populations in stored franks except in one case. Aerobic cell populations detected on frankfurters receiving N + SL + SD and HDP treatments and stored for 7 and 28 days (3.50 and 3.30 log<sub>10</sub> cfu/g, respectively) were significantly lower than the aerobic populations detected on franks receiving only HDP treatment.

## DISCUSSION

Results from our MIC studies showed that SL at 6% concentration in broth was not effective in preventing *L. monocytogenes* growth. We used higher SL concentration (10%) as a dipping treatment to obtain meaningful bactericidal effect (data not shown). Our results are in agreement with Shelef and Yang (1991) who reported delayed growth of three *L. monocytogenes* strains in TSB containing 7.86% SL. In another study, Uhart *et al.* (2004) found that 6% SL in TSB was not inhibitory to four strains of *L. monocytogenes*. Our results of MICs of 0.4% SD for *L. monocytogenes* were also supported by the findings of Uhart *et al.* (2004). They found MICs of 0.4–0.5% among the four *L. monocytogenes* strains tested. Mohamed *et al.* (1984) obtained complete inhibition of *L. monocytogenes* at 32 or 256-IU N/mL depending upon the strain tested. Inhibitory activity of N varied with five strains of *L. monocytogenes*, with zones of inhibition in a well assay ranging from 6 to 20 mm (Vignolo *et al.* 2000). The presence of N in TSB extended the lag phase of *Listeria monocytogenes* L62 strain by 5 days at 7C compared with 16-days extension of lag phase for *Listeria monocytogenes* L99 strain (Dykes and Moorhead 2000).

The inhibitory effect of the antimicrobials used in this study was not dependent on pH reduction because the frankfurter pH remained at approximately 5.8 throughout the 28-day storage period. The slight increase in frank-

Further pH during storage may have occurred because of the buffering capacity of the meat components (Van Netten *et al.* 1994). Similar effects were reported in studies with other RTE meat products (Mbandi and Shelef 2001; Stekelenburg and Kant-Muermans 2001; Mbandi and Shelef 2002). Barmपालia *et al.* (2004) reported that frankfurters treated with SD had a lower pH compared with franks treated with lactic acid. HDP treatment did not alter the pH of meat samples.

### **Antilisterial Effect of Antimicrobials**

N was more bactericidal to *L. monocytogenes* than SL or SD. Further, combinations of N with SL and/or SD also exerted greater *L. monocytogenes* reduction in frankfurters than SL or SD alone. Glass *et al.* (2002) found that *L. monocytogenes* populations in wieners stored for 45 days at 4.5C were ca. 2 log lower when treated with SL + SD than the wieners treated with SL or SD alone. Our results are in agreement with Samelis *et al.* (2001) who reported that 10% SL or 5% SD dipping treatment of pork bologna did not significantly reduce *L. monocytogenes* during storage. Further, they found frankfurter formulations with a combination of SL and SD that showed greater *L. monocytogenes* reduction than the formulations with SL alone during storage for 120 days at 4C (Samelis *et al.* 2002). The presence of SD in frankfurter formulations might have contributed to *L. monocytogenes* reduction in control samples.

N has been studied to control spoilage and pathogenic bacterial growth in RTE meats (Davies *et al.* 1999; Gill and Holley 2000). The antilisterial action of N occurs immediately after cells are exposed to the bacteriocin resulting in cell death (El-Khateib *et al.* 1993). Synergistic inhibition of *L. monocytogenes* on salmon was reported using N and SL (Nykanen *et al.* 2000). Uhart *et al.* (2004) observed that combinations of SL, SD and pediocin were more effective at reducing populations of some strains of *L. monocytogenes* in beef franks than the SL + SD treatments. The antilisterial effect of N in raw beef was enhanced when the bacteriocin was combined with lactate; however, the reduction due to this combination was not significantly different than using N alone (Ariyapitipun *et al.* 2000).

### **Effect of HDP on *L. monocytogenes***

HDP treatment, primarily studied for tenderness improvement in muscle food products, has been evaluated as a nonthermal treatment to inactivate bacterial populations in meats. With limited studies, the bactericidal role of HDP is not clear. Researchers have shown a significant reduction in the total aerobic populations in ground beef and stew pieces (Williams-Campbell and Solomon 2000, 2002) and in intact beef muscle (Schilling *et al.* 2003).

However, Lorca *et al.* (2002) did not find a significant effect of HDP treatment on the natural bacterial flora of ground beef. HDP treatment resulted in ca.  $0.5 \log_{10}$  cfu/g reduction of *L. monocytogenes* on beef muscle (Patel *et al.* 2005). The shock waves generated during HDP treatments are in the range of 70–100 MPa for fractions of milliseconds (Williams-Campbell and Solomon 2002). In our study, HDP treatment alone reduced *L. monocytogenes* populations by ca.  $1 \log_{10}$  cfu/g. Other pressure treatments have been used in combination with antimicrobial treatments to enhance bactericidal effects. HPP has been studied as a postlethal treatment in RTE meats (Lucore *et al.* 2000; Hayman *et al.* 2004). *Listeria monocytogenes* inactivation in frankfurters by HPP was influenced by salt content (Hayman *et al.* 2004). The bactericidal efficacy of N was enhanced under high pressure (Masschalck *et al.* 2001). As with HPP, HDP treatment may be combined with other antimicrobials to enhance its bactericidal effects.

HPP resulted in the lower recovery of pathogenic bacteria on selective media (Kalchayanand *et al.* 1994). The inability of some of the cells surviving a treatment to form colonies on a selective medium while retaining the ability to form colonies on a nonselective medium is due to sublethal injury. These injured cells are sensitive to bacteriocins and subsequently die in the presence of antimicrobials (Ray 1993). In our study, relatively higher populations of *L. monocytogenes* were detected on nonselective TSAYE medium compared with the population detected on selective MOX media. The pressure fronts generated during HDP treatment could have sensitized injured bacteria to N in a manner similar to HPP (Masschalck *et al.* 2003).

From the results of our study, *L. monocytogenes* clearly survived at 4C for 4 weeks in the presence of organic acids and N, necessitating a storage temperature of less than 4C to prevent *Listeria* survival and/or growth. N and HDP may potentially be used to prevent postprocessing contamination of RTE meat products with *L. monocytogenes*. From our results, N is superior to SL or SD in reducing *L. monocytogenes* on frankfurters, and its effect was significantly enhanced when used in combination with HDP. Further, there is a potential for cells to develop resistance to N during prolonged exposure. Such problem could be avoided with other antimicrobial interventions such as HDP treatment. Using a postprocessing treatment like HDP coupled with adequate manufacturing and sanitary practices can enhance the safety of RTE meats.

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