

# Application of Antimicrobials as Post-Processing Solutions to Control *Listeria monocytogenes* on Sliced Pork Bologna Stored at 4°C in Vacuum Packages

M.L. Kain, J. Samelis, J.N. Sofos, J.A. Scanga, K.E. Belk and G.C. Smith

## SUMMARY

Post-processing contamination of cured meats with *Listeria monocytogenes* has become a major safety concern and threat to the meat processing industry worldwide. Effective treatments are necessary to inhibit post-processing contamination of *L. monocytogenes* in cured pork and other meat products. This study evaluated the effectiveness of single antimicrobials or combinations of antimicrobials applied as dipping solutions on the growth of inoculated *L. monocytogenes* on pork bologna after slicing and before vacuum packaging and storage. Dipping solutions of acetic acid, lactic acid, potassium benzoate, potassium sorbate and sodium diacetate applied singly and/or in combinations with nisin appeared to have the most propensity for controlling the growth of *Listeria monocytogenes* and for potential application in the meat industry.

**Key words:** bologna, antimicrobials, *Listeria monocytogenes*

## INTRODUCTION

Following the recent listeriosis outbreak, which caused the unfortunate death of 21 individuals and at least 100 illnesses in 14 states due to the consumption of post-processing contaminated hot dogs and luncheon meats (CDC, 1999), *L. monocytogenes* has re-emerged as a pathogen of concern in the USA. Since technologies such as irradiation are not approved for use on packaged, ready-to-eat products, interest in the incorporation of chemical (e.g., lactates, acetates, sorbates) or biological (e.g., bacteriocins) antimicrobial compounds (Yen *et al.*, 1991; El-Khateib *et al.*, 1993; Wederquist *et al.*, 1994; Blom *et al.*, 1997; Murray and Richard, 1997;

Kuntz, 1999) as a safety barrier has been renewed.

## MATERIALS AND METHODS

**Preparation of pork bologna.** Fresh pork trimmings of approximately 30% fat were obtained from Swift Company (Greeley, CO). Bologna ingredients consisted of (% w/w): pork trimmings (82.2), water as ice (10), dextrose (2), corn syrup solids (2), sodium chloride (2), dry mustard (0.9), phosphate (sodium tripolyphosphate and sodium hexametaphosphate, Heller, Inc., Bedford Park, IL) (0.4), sodium nitrite (0.0156), sodium erythorbate (0.05), paprika (0.25), onion powder (0.05), garlic powder (0.05), coriander (0.05) and white pepper (0.05). Raw pork trimmings and all other ingredients were emulsified in a Meissner 35 L bowl chopper (RMF, Kansas City, MO) at high speed for 3-5 minutes (3,000 rpm blade speed, 18 rpm bowl speed). The final temperature of the bologna batter after mixing was 5°C. The batter was extruded into 65 mm diameter, fibrous cellulose casings (Koch, Kansas City, MO) and weighed. The bologna was cooked in a smokehouse (Alkar, Lodi, WI) to a final core temperature of 70°C (155°F). After cooking, the bologna was showered for 5 min with cool tap water and stored at 4°C overnight. The next day, the casings were removed manually and the bologna was sliced approximately 5 mm thick with a Globe slicer (Mozley Manufacturing, Stamford, CT) to an average slice weight of approximately 25 g. Slices were wrapped in meat wrapping paper and transferred to the microbiology laboratory for inoculation, treatment, vacuum packaging, storage, and analysis.

**Preparation of *Listeria monocytogenes* inoculum.** A *L. monocytogenes* composite was prepared from ten strains: one human isolate, four pork sausage isolates, one pork meat isolate, and four pork variety meat isolates. Each strain was activated by transferring 0.1 ml of the frozen culture to 10 ml of sterile Tryptic Soy Broth with 0.6% Yeast Extract (TSBYE) (Becton Dickinson, Sparks, MD) and incubated at 30°C for 24 h. Strains were subcultured twice in TSBYE before use in the experiments. After the second subculturing, the cultures of each strain were combined, centrifuged (6,000 rpm for 15 min) and washed

with sterile phosphate buffered saline (PBS). After washing, the mixed culture was serially diluted with sterile PBS to a concentration capable of giving  $10^2$ - $10^3$  CFU/cm<sup>2</sup> of inoculated product. This composite inoculum was used to inoculate the bologna slices. To confirm the desired concentration of cells, the inoculum was plated on Tryptic Soy Agar with 0.6% Yeast Extract (TSAYE) (Becton Dickinson) and PALCAM (Becton Dickinson) agars and incubated at 30°C for 24 h.

**Product inoculation.** Slices of bologna were placed on aluminum foil under a biohazard laminar flow hood and 0.25 ml of the composite inoculum was deposited on one side of a slice and spread with a sterile bent glass rod on the surface. Inoculated slices were left to stand separately at 5°C for 15 min for inoculum attachment. The same procedure was repeated for the other side of each slice. The target inoculation level of bologna to evaluate post-processing decontamination solutions of antimicrobials was  $10^2$ - $10^3$  CFU/cm<sup>2</sup>.

**Treatments of bologna with antimicrobials and microbiological analysis.** Inoculated bologna slices were immersed in different sterile antimicrobial solutions mixed in distilled water. This experiment was designed to optimize effective concentrations of organic acids or their salts as single antimicrobials in dipping solutions to control *L. monocytogenes*, without adversely affecting the sensory quality of cured pork products. Included were treatments of acetic acid (2.5 and 5%), lactic acid (2.5 and 5%), sodium acetate (2.5 and 5%), sodium diacetate (2.5 and 5%), sodium lactate (5 and 10%), potassium benzoate (5%), potassium sorbate (5%), nisin (0.5%) and nisin in combination with organic acids or their salts. Each slice of bologna was transferred from the aluminum foil with sterile forceps and placed in the sterile dipping solution for 1 min. Immediately after dipping, two slices per sample were inserted into a vacuum bag (15 x 22 cm, 3 mil std barrier, Nylon/PE vacuum pouch; Koch, Kansas City, MO), vacuum packaged (Multivac, Germany) at 80 mm Hg and stored at 4°C for 90 days. Samples were analyzed immediately after inoculation (day 0) and after 10, 20, 35, 50, 70 and 90 days to enumerate *L.*

*monocytogenes* and total bacterial counts.

For microbiological analysis, samples were transferred into individual sterile stomacher bags (Whirl-Pak®, Nasco), mixed with 100 ml of 0.1% buffered peptone water (Becton Dickinson) and shaken 30 times, as described in the United States Meat and Poultry Inspection Regulation (FSIS, 1996). On each testing day, three samples per treatment were analyzed and appropriate serial decimal dilutions were made and then plated by spreading 0.1 ml on duplicate agar plates. Two media types were used: TSAYE (non-selective) and PALCAM agar (selective). Colonies on all plates were counted after incubation at 30°C for 48 h. The lowest detection limit of the analysis was 0.9 log CFU/cm<sup>2</sup>, calculated as follows: the lowest detectable number of bacteria in the homogenized sample was 10 CFU/ml, i.e., 1 colony on each plate after spreading of 0.1 ml directly from the bag, thus, 1,000 colonies were present in the bag (100 ml). This number was divided by the total bologna surface (33 cm<sup>2</sup>/side x 2 sides/slice x 2 slices/bag = 132 cm<sup>2</sup>) to result in 8 CFU/cm<sup>2</sup>, i.e. 0.9 log CFU/cm<sup>2</sup>. Also the pH (Accumet 50, Fisher Scientific) of each sample was determined by immersing the pH electrode (Denver Instruments, Arvada, CO) in the stomacher bag after samples were plated.

## RESULTS

Bacterial growth on PALCAM agar from inoculated slices without treatment exceeded 6 log CFU/cm<sup>2</sup> at 20 d (Tables 1-3). No increases ( $P > 0.05$ ) in populations occurred on product treated with 5% acetic acid, 5% sodium diacetate and 5% potassium benzoate from day 0 to 90, while product treated with 5% lactic acid or 5% potassium sorbate was stored for 70 to 90 days before an increase ( $P < 0.05$ ) in bacterial populations occurred (Table 1). At a concentration of 2.5% in the dipping solution, only acetic acid maintained its antilisterial activity throughout storage, while lower concentrations of lactic acid (2.5%), sodium diacetate (2.5%) and sodium acetate (2.5%) (data not shown) and sodium acetate (5%) and sodium lactate (5 and 10%) permitted bacterial growth at earlier days of storage (Table 1). The antimicrobials effective against *L.*

*monocytogenes* also inhibited total bacterial growth on TSAYE (data not shown). This indicated that dipping or spraying of certain antilisterial compounds may help to extend the microbiological shelf-life of sliced, cooked, vacuum-packaged meats.

Nisin was combined with organic acids or their salts as post-processing solutions in an attempt to increase their antilisterial effect in the form of multiple hurdles. The concentrations of organic acids or salts in dipping solutions were similar to those that inhibited, retarded or permitted growth of the pathogen, as single antimicrobials. Nisin (0.5%) used alone, had an immediate listericidal effect at day 0 (Table 1), however when combined with acetic and lactic acids, it demonstrated similar results to those of the organic acid dipping solutions of similar concentrations without nisin. Nisin combined with 3 and 5% acetic acid showed inhibition of *L. monocytogenes* throughout 90 days of storage, while only 5% lactic acid demonstrated similar inhibitory effects though not as significant (Table 2). These results proved that lower concentrations of acetic acid combined with nisin are capable of producing similar effects compared to the higher acetic acid concentrations, while higher concentrations of lactic acid are still required to maintain an inhibitory effect of *L. monocytogenes* through 90 days of storage. The combination of nisin with salts of organic acids in dipping solutions did not alter the antilisterial effect of these salts, but increased the magnitude and duration of this effect when compared to dipping solutions of similar salt concentrations without nisin. Therefore, the antilisterial effect of salts was increased due to nisin supplementation, especially that of the less effective salts singly, such as sodium acetate (Table 3). However, as with lactic acid, lower concentrations (3%) of sodium acetate and sodium diacetate when combined with nisin were less effective than the higher concentrations (5%) of the same salts combined with nisin.

The pH of bologna was reduced after treatment (day 0) with lactic and acetic acids and sodium diacetate. This immediate pH reduction was greater with increases (from 2.5 to 5%) of the acid or sodium diacetate concentration; often, a pH of approximately 5.0 or

lower was measured after dipping of bologna in the 5% solutions, while at the same time the control samples had a pH of 1-1.5 units higher (6.2-6.4) (data not shown). Although the buffering capacity of the product tended to restore the original pH with time, product values remained below 6.0 in several of the treatments throughout refrigerated (4°C) storage, and in the absence of significant growth of the pathogen or the natural flora in the packs. In contrast to the use of organic acids and sodium diacetate, all other antimicrobials did not have major effects on product pH after immersion and throughout storage (data not shown). Sensory evaluation should be carried out before commercial application of post-processing decontamination treatments of cured meats with any of these chemicals.

## IMPLICATIONS

The most promising antimicrobials used as dipping solutions for controlling growth of *L. monocytogenes* on sliced bologna, with potential use in the meat industry, were acetic acid, lactic acid, potassium benzoate, potassium sorbate and sodium diacetate applied singly and/or in combinations with nisin. Notably, nisin alone and sodium lactate as dipping solutions had very limited antilisterial effects. Increasing the level of chemical preservatives in cured pork products in an attempt to inhibit *L. monocytogenes* can be avoided by incorporating more antimicrobials and/or decontamination procedures in combinations which may lessen any negative effects on the sensory quality of the final product. The results of this study are valuable to the pork processing industry as it seeks measures and modified procedures to protect consumers from the deadly pathogen *L. monocytogenes*, and will be beneficial the pork producers by increasing product safety and consumer confidence in pork products.

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**Table 1.** Populations (log cfu/cm<sup>2</sup> ± SD; n=9) of inoculated *L. monocytogenes* (PALCAM agar) on bologna slices immersed for 1 min in antimicrobial solutions, vacuum packaged and stored at 4°C.

Treatments/Dipping solutions	Days of storage at 4°C						
	0	10	20	35	50	70	90
Uninoculated: No treatment	<0.9 (0.0)	<0.9 (0.0)	0.9 (0.1)	<0.9 (0.0)	0.9 (0.3)	1.4 (0.8)	1.6 (0.8)
Inoculated: No treatment	2.2 (0.4)	3.8 (1.2)	7.4 (0.5)	7.6 (0.4)	8.0 (0.2)	8.0 (0.2)	8.0 (0.2)
Uninoculated: Water immersion	<0.9 (0.0)	<0.9 (0.0)	1.4 (0.5)	0.9 (0.1)	1.1 (0.8)	1.4 (0.8)	1.5 (0.9)
Inoculated: Water immersion	1.9 (0.5)	4.7 (0.8)	7.4 (0.1)	7.6 (0.5)	7.9 (0.2)	7.8 (0.3)	7.7 (0.2)
Acetic acid (5%)	1.8 (0.5)	1.4 (0.6)	1.2 (0.6)	1.2 (0.9)	1.4 (0.4)	1.5 (0.8)	1.1 (0.5)
Lactic acid (5%)	1.5 (0.5)	1.2 (0.6)	2.4 (1.0)	2.4 (2.0)	2.7 (1.2)	1.4 (2.0)	4.5 (1.5)
Sodium acetate (5%)	1.7 (0.4)	2.2 (1.1)	4.0 (2.5)	5.1 (0.9)	6.5 (0.6)	7.4 (0.2)	7.3 (0.8)
Sodium diacetate (5%)	1.7 (0.5)	1.5 (0.6)	1.6 (0.5)	3.2 (2.2)	2.7 (1.7)	2.7 (1.2)	2.9 (1.7)
Potassium benzoate (5%)	2.1 (0.4)	1.9 (0.6)	1.6 (0.5)	2.2 (0.8)	1.9 (0.4)	3.2 (1.0)	1.0 (1.7)
Potassium sorbate (5%)	2.0 (0.4)	2.4 (0.3)	2.1 (1.1)	2.7 (0.6)	3.9 (0.5)	5.0 (1.0)	5.8 (2.0)
Sodium lactate (5%)	1.8 (0.2)	3.8 (1.3)	6.6 (1.1)	6.7 (1.6)	7.7 (0.5)	8.1 (0.3)	7.3 (0.4)
Sodium lactate (10%)	2.0 (0.4)	3.8 (0.5)	4.6 (1.8)	7.1 (0.8)	8.0 (0.4)	7.9 (0.3)	7.2 (0.4)
Nisin (0.5%)	<1.9	1.0 (0.2)	>6.3	7.8 (0.1)	7.0 (0.1)	N/A	N/A

**Table 2.** Populations (log cfu/cm<sup>2</sup> ± SD; n=3) of inoculated *L. monocytogenes* (PALCAM agar) on bologna slices immersed for 1 min in antimicrobial solutions, vacuum packaged and stored at 4°C.

Treatments/Dipping solutions	Days of storage at 4°C						
	0	10	20	35	50	70	90
Uninoculated: No treatment	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	0.9 (0.0)
Inoculated: No treatment	2.5 (0.4)	4.2 (0.3)	6.3 (0.1)	7.6 (0.1)	7.9 (0.3)	7.5 (0.1)	7.5 (0.2)
Uninoculated: Water immersion	<0.9	<0.9	2.3 (0.3)	1.8 (0.8)	<0.9	<0.9	0.9 (0.0)
Inoculated: Water immersion	2.9 (0.0)	3.4 (1.2)	6.6 (0.3)	7.4 (0.2)	7.1 (0.1)	6.8 (0.3)	6.6 (0.1)
Lactic acid (3%) with nisin (0.5%)	<1.9	0.9 (0.1)	4.5 (0.7)	6.0 (0.4)	5.8 (0.6)	7.3 (0.1)	7.3 (0.1)
Lactic acid (5%) with nisin (0.5%)	1.9 (0.0)	1.0 (0.2)	1.2 (0.4)	0.9 (0.0)	1.5 (0.8)	5.3 (0.6)	1.9 (0.0)
Acetic acid (3%) with nisin (0.5%)	1.0 (0.2)	1.1 (0.2)	0.9 (0.1)	1.3 (0.1)	0.9 (0.0)	0.9 (0.1)	0.9 (0.0)
Acetic acid (5%) with nisin (0.5%)	1.6 (0.3)	1.9 (0.0)	<0.9	<0.9	0.9 (0.0)	0.9 (0.0)	0.9 (0.0)

**Table 3.** Populations (log cfu/cm<sup>2</sup> ± SD; n=3) of inoculated *L. monocytogenes* (PALCAM agar) on bologna slices immersed for 1 min in antimicrobial solutions, vacuum packaged and stored at 4°C.

Treatments/Dipping solutions	Days of storage at 4°C						
	0	10	20	35	50	70	90
Uninoculated: No treatment	<0.9	<0.9	<0.9	<0.9	0.9 (0.1)	<0.9	0.9 (0.0)
Inoculated: No treatment	<1.9	4.2 (0.1)	6.2 (0.2)	7.9 (0.3)	7.7 (0.1)	8.2 (0.2)	7.9 (0.1)
Uninoculated: Water immersion	<0.9	<0.9	<0.9	<0.9	0.9 (0.0)	1.4 (0.5)	0.0 (0.0)
Inoculated: Water immersion	2.7 (0.2)	4.9 (0.1)	6.6 (0.3)	7.6 (0.2)	7.6 (0.2)	7.7 (0.1)	7.4 (0.2)
Potassium benzoate (3%) with nisin (0.5%)	<0.9	<0.9	0.9 (0.0)	<1.9	1.7 (0.7)	1.3 (0.7)	2.4 (0.6)
Potassium sorbate (3%) with nisin (0.5%)	1.2 (0.4)	<1.9	1.9 (0.0)	2.0 (0.2)	3.2 (0.1)	4.0 (0.4)	5.6 (0.1)
Sodium acetate (3%) with nisin (0.5%)	0.9 (0.1)	1.9 (0.0)	2.4 (0.4)	3.6 (0.6)	5.8 (0.1)	7.4 (0.2)	7.5 (0.1)
Sodium acetate (5%) with nisin (0.5%)	<0.9	1.9 (0.0)	1.9 (0.0)	3.2 (0.2)	3.7 (0.5)	5.1 (1.2)	6.0 (1.0)
Sodium diacetate (5%) with nisin (0.5%)	1.1 (0.2)	1.1 (0.3)	0.9 (0.0)	0.9 (0.0)	0.9 (0.0)	0.9 (0.0)	0.9 (0.0)