RESEARCH REPORT
PORK SAFETY

Title: Optimization of Antimicrobials for Control of *Listeria monocytogenes* and for Acceptable Pork Product Quality **- NPB #02-002**

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Abstract: Contamination of ready-to-eat meat products with *Listeria monocytogenes* is a major concern to the meat processing industry and needs to be addressed in order to enhance the safety of these products. This report describes a number of studies with this goal in mind. Two studies evaluated the effectiveness of antimicrobials, applied singly and in combination in the formulation of bologna and frankfurters against *L. monocytogenes* inoculated post-processing as well as spoilage microorganisms found naturally on the products, during storage in vacuum packages at 4°C (bologna) and 10°C (bologna and frankfurters). Furthermore, the antilisterial effect of additives in the formulation followed by immersion of frankfurters into organic acid solutions was determined. In a third study, Nisaplin®, organic acids and a preservative applied as dipping solutions either singly or sequentially to control post-processing *L. monocytogenes* contamination of commercial bologna and ham stored at 10°C in vacuum packages was investigated. The effect of additives in the formulation and dipping solutions on the sensory qualities of frankfurters (formulated in our laboratory and commercial) and commercial bologna and ham was also determined. Finally, the acid tolerance response of the pathogen on stored, inoculated frankfurters formulated and/or treated with organic acids, to synthetic gastric fluid, was investigated.

Results showed that combinations of antimicrobials in the formulation of bologna and frankfurters suppressed the growth of *L. monocytogenes* more effectively during storage at 4°C (bologna) and 10°C (bologna and frankfurters) than antimicrobials applied singly. Sodium lactate (1.8%) combined with sodium diacetate (0.25%) was found to be the most effective combination in the formulation of bologna and frankfurters. Frankfurters formulated with sodium lactate (1.8%) and sodium diacetate (0.125% and 0.25%) and treated with lactic acid (2.5%) or acetic acid (2.5%) for 2 min resulted in reductions of the pathogen during storage at 10°C. Dipping of commercial bologna and ham in acetic acid (2.5%), lactic acid (2.5%) or potassium benzoate (5%), applied singly or sequentially with Nisaplin (0.5%) for 2 min, also reduced pathogen levels during storage at 10°C. The combination treatments, however, were more effective in reducing *L. monocytogenes* since they caused higher initial reductions. Sensory qualities of commercial bologna and ham treated with acetic acid, lactic acid and potassium benzoate treatments resulted in lower odor, flavor and overall acceptability scores as compared to the controls.

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The most effective formulation combination treatment (1.8% sodium lactate + 0.25% sodium diacetate) did not seem to affect the flavor and overall acceptability of frankfurters. Furthermore, dipping of commercial frankfurters in acetic acid, lactic acid or potassium benzoate had no apparent effect on their sensory qualities. Finally, under the conditions of this study, the acid tolerance response of *L. monocytogenes* to synthetic gastric fluid appeared to increase during storage of frankfurters formulated with 0.25% sodium diacetate and dipped in 2.5% lactic acid. The results of this study should be useful to the meat industry in their efforts to inactivate or inhibit the growth of this deadly pathogen on ready-to-eat meat products during storage.

Introduction: *Listeria monocytogenes*, the causative agent of listeriosis has been associated with frequent and highly publicized recalls of ready-to-eat meats contaminated with this pathogen. A listeriosis outbreak caused by the consumption of post-processing contaminated cured meat products caused 21 deaths and more than 100 illnesses in 14 states in 1998-1999 (CDC, 1999). More recently, sliceable turkey deli meat was linked to another multistate outbreak that caused 46 illnesses, 7 deaths and 3 stillbirths or miscarriages, and was followed by a recall of 27.4 million pounds of product (CDC, 2002).

Listeria monocytogenes is ubiquitous and has the ability to colonize meat plants and survive under unfavorable conditions (Lou and Yousef, 1999). Processing, sanitation strategies, and HACCP programs applied in the meat industry appear to often be insufficient to prevent the presence or inhibit the growth of the pathogen in processed meats (Tompkin et al., 1999; Tompkin, 2002). Products may be recontaminated after cooking; thus there is a need to develop post-processing hurdle technologies to inactivate or inhibit the growth of *L. monocytogenes* in meat products.

Previous studies in our laboratory tested the antilisterial activity of antimicrobials in the formulation of vacuum packaged pork frankfurters stored at 4^{o}C. The antimicrobials (sodium acetate, sodium diacetate, sodium lactate) were either used singly (Bedie et al., 2001) or in combinations of two (Samelis et al., 2002) and different concentrations were evaluated for their effectiveness. Results showed that post-processing contamination on frankfurters can be controlled by lower (1.8%) than permissible (3%) levels of sodium lactate in combination with permissible (0.25%) levels of sodium acetate, sodium diacetate, or glucono-delta-lactone included in the formulation (Samelis et al., 2002). Furthermore, a post-packaging thermal treatment (80°C, 60 s) was found to enhance the antilisterial effect of the additivies (Samelis et al., 2002). In another study, *L. monocytogenes* was controlled in pork bologna by dipping of the products in solutions of acetic acid (2.5–5%), lactic acid (5%), sodium diacetate (5%) and potassium benzoate (5%) (Samelis et al., 2001). Effective antimicrobial treatments for post-processing contamination with *L. monocytogenes* were thus identified in these studies. Their antimicrobial activity, however, during storage of the products at abusive storage temperatures (10°C) and their effects against spoilage microorganisms that occur naturally in the product were not determined. Furthermore, their activity on commercially prepared products and effects on product quality/palatability need to be evaluated.

Objectives: The objectives of this project are to evaluate the antilisterial activity of antimicrobials, applied in the formulation of pork products or as dipping solutions, during storage at 10^{o}C (to simulate potential abuse at retail) and to optimize combinations and concentrations, in order to maximize bactericidal effects with minimal negative effects on the sensory quality of the products. Questions to be answered include: (a) Which

combinations and concentrations of antimicrobials are effective against *L. monocytogenes*? (b) Do chemical or physical characteristics of the products affect the effect of antimicrobials? (c) How can the negative effects of the additives on the sensory characteristics of the products be minimized?

Materials and Methods:Study 1: Antimicrobials in the formulation of pork bologna for control of *Listeria monocytogenes* inoculated after slicing and stored at 4°C and 10°C in vacuum packages.

Nine treatments were evaluated for their effectiveness against the pathogen during storage of inoculated bologna under vacuum at 4°C and 10°C. The bologna formulation consisted of (%, wt/wt): pork meat (approximately 30% fat; 82.2), ice (10), sodium chloride (2), dextrose (2), dry mustard (0.9), corn syrup solids (2), polyphosphate (sodium tripolyphosphate and sodium hexameta-phosphate; 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). Spices and seasonings were purchased from AC Legg Co. (Birmingham, AL). Nine batches were formulated separately to contain:

1. No antimicrobials (control)
2. Sodium lactate (3% of a 60% [wt/wt] commercial product which equals to 1.8% pure sodium lactate, Purac Inc., Lincolnshire, IL)
3. Sodium diacetate (0.125%, Niacet, Niagara Falls, NY)
4. Glucono-D-lactone (0.125%, GDL, Sigma Chemical Co., St. Louis, MO)
5. Sodium lactate (1.8%) combined with sodium diacetate (0.125%)
6. Sodium lactate (1.8%) combined with GDL (0.125%)
7. Sodium lactate (1.8%) combined with sodium diacetate (0.25%)
8. Sodium lactate (1.8%) combined with GDL (0.25%)
9. Sodium lactate (1.8%) combined with sodium diacetate (0.125%) and GDL (0.125%).

The ingredients of each batch were emulsified in a bowl chopper (RMF, Kansas City, MO) for 3-5 min to a final temperature of 15.5°C. The mixture was then extruded (Handtmann Inc., Buffalo Grove, IL) into 65 mm diameter fibrous cellulose casings (Koch, Kansas City, MO). The bologna was weighed and then cooked in dry air for 1 hour (smokehouse temperature 60°C), followed by hot smoking (liquid smoke) for 38 min. After smoking, the bologna was cooked with steam for 1 hour (smokehouse temperature 71°C, relative humidity 50%). The smokehouse temperature was then increased to 88°C and the bologna was cooked until its internal temperature reached 70°C. After cooking, the bologna was showered with cool tap water for 5 min, cooled overnight at 4°C and reweighed for cooking yield determination. The casings were then removed and the bologna was sliced into approximately 5 mm thick slices with a Globe slicer (Mozley Manufacturing, Stamford, CT).

The slices were inoculated (3-4 log CFU/cm^2) on both sides with a mixture of 10 *L. monocytogenes* strains. The strains used in this study included Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b) and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate) and PVM1, PVM2, PVM3 and PVM4 (pork variety meat isolates, serotype not known). All the strains were available as frozen stock cultures (-70°C) and were activated by transferring a loopful of stock culture into 10 ml of Tryptic Soy broth (Difco) with 0.6% yeast extract (Acumedia, Baltimore, MD; TSBYE) and incubating at 30°C for 24 h. The strains were subcultured twice in TSBYE before use in the experiment. The

TSBYE cultures were centrifuged (five strains in each conical centrifuge tube; 6,000 rpm, 15 min, 4°C), washed with 10 ml of sterile phosphate-buffered saline (PBS) and then centrifuged again (6,000 rpm, 15 min, 4°C). The resulting pellet was resuspended with 20 ml PBS and serially diluted to a concentration estimated to yield 3-4 log CFU/cm^2 of bologna. The slices were placed on sterile pieces of aluminum foil and were inoculated on one side (0.1 ml from the appropriate dilution) under a biological safety cabinet. The inoculum was spread over the entire surface with a sterile bent glass rod. The inoculated slices were left to stand for 15 min at 5°C for attachment and then the above procedure was repeated for the other side.

After inoculation two bologna slices per treatment were vacuum packaged (bags of 15 by 20 cm, 3 mil std barrier, Nylon/PE vacuum pouch, Koch) and stored at 4°C or 10°C. Microbiological and pH determinations (three replicates, two samples per sampling time and treatment) were conducted on all samples on days 0, 10, 20, 35, 70 and 90 for samples stored at 4° C, and on days 0, 4, 8, 12, 16, 20, 24 and 28 for samples stored at 10°C. Total microbial populations were enumerated on Tryptic Soy Agar (TSA, Difco) with 0.6% yeast extract (TSAYE; 25°C for 72 h), *L. monocytogenes* on PALCAM agar (Difco; 30°C for 48 h), lactic acid bacteria (LAB) on de Man Rogosa Sharpe agar, pH 5.5 (MRS; International BioProducts, Bothell, WA; 25°C for 72 h, aerobic atmosphere), *Pseudomonas* sp. on Pseudomonas Agar (Oxoid Ltd, Basingstoke, Hampshire, England; 25°C for 72 h) supplemented with cetrimide-fucidin-cephalosporin (Oxoid), *Brochothrix thermosphacta* on Streptomycin-Thallous Acetate-Actidione agar (STAA; the medium was made from basic ingredients in the laboratory; Gardner, 1966; 25°C for 72 h), *Enterobacteriaceae* on Violet Red Bile Glucose agar (Acumedia; 35°C for 24 h), and yeasts and molds on Rose Bengal agar (Difco; 25°C for 72 h).

Study 2 :Control of *Listeria monocytogenes* with antimicrobials in the formulation and by dipping in organic acids of post-processing inoculated pork frankfurters stored at 10°C in vacuum packages.

The ingredients used in the basic formulation of the pork frankfurters were the same as those used in Study 1. Five batches were prepared separately in order to contain:

1. No antimicrobials (control)
2. Sodium lactate (3% of a 60% [wt/wt] commercial product, which equals to 1.8% pure sodium lactate, Purac Inc., Lincolnshire, IL)
3. Sodium diacetate (0.25%, Niacet, Niagara Falls, NY)
4. Sodium lactate (1.8%) combined with sodium diacetate (0.25%)
5. Sodium lactate (1.8%) combined with sodium diacetate (0.125%).

The ingredients were emulsified as in the previous study and the batters were extruded into 24 mm diameter cellulose casings (Koch). The frankfurters were then weighed and cooked in dry air for 30 min (smokehouse temperature 80°C), followed by hot smoking (liquid smoke) for 30 min. The frankfurters were cooked with steam for 30 min (smokehouse temperature 80°C, relative humidity 26%), showered with cool tap water for 5 min and cooled overnight at 4°C. The following day the frankfurters were reweighed for cooking yield determination, peeled manually and cut into 10 cm length pieces. The frankfurters were then transferred to the microbiology laboratory for inoculation, treatment with organic acid solutions, vacuum packaging, storage and analysis.

The *L. monocytogenes* strains used in this study and the inoculum preparation were the same as those in Study 1. However, the mixed culture was resuspended in

100 ml PBS and then serially diluted to a concentration estimated to yield 2-3 log CFU/cm^2 of frankfurters when 0.25 ml of the inoculum was applied to each frankfurter. Two frankfurters from each treatment were transferred into vacuum bags (15 by 20 cm, 3 mil std barrier, Nylon/PE vacuum pouch, Koch) and inoculated (0.25 ml from the appropriate dilution applied on each frankfurter) under a biological safety cabinet. The frankfurters were then massaged in order to spread the inoculum uniformly over the entire surface. The inoculated frankfurters were left to stand for 15-30 min at 5^{o}C for attachment before treating with organic acid solutions and/or vacuum packaging. The organic acids used were 2.5% lactic acid (LA, Sigma, St. Louis, MO) and 2.5% acetic acid (AA, Mallinckrodt and Baker, Paris, KY). The frankfurters to be dipped were removed from the bags in which they were inoculated, immersed in the solutions and stirred occasionally for 2 min. They were then removed, vacuum packaged (two frankfurters per bag) and stored at 10^{o}C.
Microbiological and pH determinations (two replicates, three samples per sampling time and treatment) were conducted on days 0, 4, 8, 12, 20, 28 and 40. The samples were analyzed for total microbial populations (TSAYE agar), *L. monocytogenes* (PALCAM agar), yeasts and molds (RBC agar), and lactic acid bacteria (MRS agar, pH 5.5).

Study 3: Nisaplin®, organic acids and a preservative applied as dipping solutions to control post-processing *Listeria monocytogenes* contamination of commercial bologna and ham stored at 10°C in vacuum packages.

Commercially prepared bologna and ham were obtained from a major processor, and were used for experiments within five days of production. Prior to inoculation of the products, bologna slices of 3 mm thickness were cut in half (58 cm^2), while ham slices of 1-2 mm thickness were cut into 8 x 5 cm pieces (40 cm^2). Slices were placed on aluminum-covered trays and were inoculated with a mixture of 10 *L. monocytogenes* strains. The identity of the strains, as well as the method of preparation of the inoculum, are described in Studies 1 and 2. After centrifugation and washing of the 10-strain mixture, cells were resuspended in 100ml PBS and serially diluted to a concentration estimated to yield 3-4 log CFU/cm^2 after inoculation of the products. Each side of the bologna and ham slices was inoculated with 0.1ml of the diluted inoculum, as described previously (Study 1). Slices were then dipped into one of the following treatments:

1. No treatment (control)
2. 2.5% Acetic acid (AA; Mallinckrodt)
3. 2.5% Lactic acid (LA; Sigma)
4. 5% Potassium benzoate (PB, Sigma-Aldrich, Milwaukee, WI)
5. 0.5% Nisaplin® (Aplin & Barret, Dorset, England), where the concentration of nisin is *ca.* 0.0125%
6. 0.5% Nisaplin followed by 2.5% AA
7. 0.5% Nisaplin followed by 2.5% LA
8. 0.5% Nisaplin followed by 2.5% PB.

All treatments were applied for 2 min, followed by draining for approximately 1 min and then vacuum packaging (bags of 15 x 22 cm, 3 mil std barrier, Nylon/PE vacuum pouch, Koch). Three slices were packaged per bag and samples were stored at 10°C for 48 days.
Microbiological and pH determinations (two replicates, three samples per sampling time and treatment) were conducted on days 0, 4, 8, 12, 20, 28, 36 and 48. The samples were analyzed for total microbial populations (TSAYE agar; 25°C for 72h) and *L.*

monocytogenes (PALCAM agar; 30°C for 48h). pH determinations were carried out on one of the three slices of each sample in distilled water (1:10 dilution).

Study 4: Sensory evaluation of frankfurters treated with antimicrobials.

Sensory evaluations were carried out on frankfurters treated and/or formulated with effective antimicrobial treatments identified from Studies 1, 2 and 3.

sensory analysis of frankfurters:

Based on results from Study 1, frankfurters were formulated to contain:

1. No antimicrobials (control)
2. Sodium lactate (1.8%) combined with sodium diacetate (0.25%)
3. Sodium lactate (1.8%) combined with sodium diacetate (0.125%) and GDL (0.125%).

Based on results from Study 2, frankfurters were formulated to contain:

1. No antimicrobials (control)
2. Sodium lactate (1.8%)
3. Sodium diacetate (0.25%)
4. Sodium lactate (1.8%) combined with sodium diacetate (0.25%)
5. Sodium lactate (1.8%) combined with sodium diacetate (0.125%).

After preparation, these frankfurters were either left undipped, or were immersed in 2.5% solutions of lactic acid (Sigma) or acetic acid (Mallinckrodt) for 2 min.
Based on results from Study 3, commercial pork frankfurters (obtained from the same major processor that provided us with the bologna and ham) were treated as follows:

1. No treatment (control)
2. 2.5 % Acetic acid (AA, Mallinckrodt); 2 min
3. 2.5% Lactic acid (LA, Sigma); 2 min
4. 5% Potassium benzoate (PB, Sigma-Aldrich); 2 min.

Frankfurters to be evaluated (formulated or commercial) were cut into 3-6 cm pieces and were either vacuum packaged or first dipped into one of the antimicrobial treatments (AA, LA or PB) for 2 min, drained and then vacuum packaged. Samples were coded with random three-digit numbers and then stored in a cooler (5°C) overnight. Sensory evaluations were performed within two days.

An untrained panel of 25 consumers was recruited from the Department of Animal Sciences at Colorado State University. A nine-point hedonic scale was used to evaluate the frankfurter's appearance, odor, flavor and overall acceptability, where 1=dislike extremely and 9=like extremely. The color (1=extremely pale; 9= extremely dark) and texture (1=extremely soft; 9= extremely firm) of the frankfurters were also evaluated. For purposes of this study, frankfurters were not heated before they were tasted, but panelists were given the option of not eating the unheated samples if they did not want to. All of the panelists, however, opted to taste the frankfurters.

Sensory analysis of bologna and ham treated with antimicrobials:

Sensory evaluations were carried out on commercial bologna and ham that were treated as follows:

1. No treatment (control)

2. 2.5 % Acetic acid (Mallinckrodt)
3. 2.5% Lactic acid (Sigma)
4. 5% Potassium benzoate (Sigma-Aldrich).

Microbiological results of Study 3 clearly showed that the combination treatments (Nisaplin followed by acetic or lactic acid, or potassium benzoate) were more effective in reducing levels of *L. monocytogenes* as compared to the single treatments. The single treatments, however, were chosen for sensory analysis since they were applied after the Nisaplin in the combination treatments. The sensory qualities of the products would thus be affected by the latter treatments (AA, LA and PB).

The antimicrobial treatments were applied to the bologna and ham slices for 2 min, followed by draining and vacuum packaging. Samples were coded with random three-digit numbers and then stored in a cooler (5°C) overnight. Sensory evaluations were performed the next day.

An untrained panel of 30 consumers was recruited from the Department of Animal Sciences at Colorado State University. A nine-point hedonic scale was used to evaluate the appearance, odor, flavor and overall acceptability of the products, where 1=dislike extremely and 9=like extremely. The color (1=extremely pale; 9= extremely dark) and texture (bologna, 1=extremely soft; 9= extremely firm; ham, 1=extremely mushy; 9= extremely chewy) of the products were also evaluated.

Study 5: Reduction of *Listeria monocytogenes* populations during exposure to a simulated gastric fluid following storage of inoculated frankfurters formulated and treated with organic acids.

Frankfurters that were prepared and stored in Study 2 were used for this study.

Exposure** of* L. monocytogenes ***to synthetic gastric fluid:

To assess the susceptibility of *L. monocytogenes* to inactivation at low pH similar to the stomach environment, use was made of a synthetic gastric fluid (8.3 g of proteose peptone, 3.5 g of D-glucose, 2.05 g of NaCl, 0.6 g of KH_2PO_4, 0.11 g of $CaCl_2$, 0.37g of KCl, 0.1 g of lysozyme, 50 mg of porcine bile and 13.3 mg of pepsin per liter of distilled water) as described previously (Beumer et al., 1992; Cotter et al., 1999; Czuprynski et al., 2002). The synthetic gastric fluid was adjusted to pH 1.0 with HCl. To test the effects of the simulated gastric environment on *L. monocytogenes* originating on the frankfurters, one inoculated frankfurter from each treatment/formulation combination was removed at 0, 10, 20, 30 and 40 days during storage at 10°C (Study 2), placed into plastic Whirlpak® bags (Nasco, Fort Atkinson, WI) in which 100 ml of synthetic gastric fluid (GF; 25ºC) was added before they were pummeled in a stomacher (IUL Instruments, Barcelona, Spain) for 2 min. Control (time-0) samples (inoculated/nontreated) were placed in bags in which 100 ml of 0.1% buffered peptone water (BPW; Difco) was added prior to pummeling. At 20-min intervals, aliquots of the samples exposed to GF (for a duration of 60 min) were removed from the bags, diluted in BPW and plated on TSAYE and PALCAM agar. The plates were incubated at 30ºC for 48 h and then enumerated for surviving populations of *L. monocytogenes.*

Results:

Study 1: Antimicrobials in the formulation of pork bologna for control of *Listeria monocytogenes* inoculated after slicing and stored at 4°C and 10°C in vacuum packages.

This study investigated the effectiveness of nine treatments (antimicrobials used singly or in combination and included in the formulation of pork bologna) against *L. monocytogenes* and spoilage organisms in bologna slices, inoculated after processing, during storage in vacuum packages at 4°C or 10°C.

The results showed that for bologna slices containing no antimicrobials (inoculated controls), *L. monocytogenes* increased from 3-4 log CFU/cm^2 to more than 8 log CFU/cm^2 in 20 days at 4°C and in 12 days at 10°C. The most effective treatment against *L. monocytogenes* was found to be 1.8% sodium lactate combined with 0.25% sodium diacetate. This treatment inhibited growth of the pathogen for 35 days at 4°C. At 10°C, however, an initial increase of 1.7 log CFU/cm^2 was observed from day-0 to day-16 followed by a decrease of 1.3 log CFU/cm^2. Sodium lactate used singly did not seem to be effective at both storage temperatures since the populations of the pathogen in 70 and 12 days at 4 and 10°C, respectively. Sodium lactate (1.8%) combined with 0.125% sodium diacetate permitted growth at 10°C. For this treatment a 4 log CFU/cm^2 increase was observed in 20 days. Also, at 4°C the populations of the pathogen exceeded 7 log CFU/cm^2 in 35 days. Sodium lactate (1.8%) combined with GDL (0.125%) was ineffective at both temperatures (*L. monocytogenes* populations reached 7 log CFU/cm^2 in 35 days at 4°C and exceeded 8 log CFU/cm^2 in 20 days at 10°C. Sodium lactate (1.8%) combined with 0.25% GDL permitted growth of 6 log CFU/cm^2 in 70 days at 4°C and 7.2 log CFU/cm^2 in 20 days at 10°C. Both sodium diacetate (0.125%) and GDL (0.125%), when used singly did not control growth of the pathogen at both temperatures. At 4°C both sodium diacetate and GDL allowed growth of 8 log CFU/cm^2 in 70 and 35 days, respectively. At 10°C, populations of *L. monocytogenes* reached 7 log CFU/cm^2 in 12 days for sodium diacetate, and 8 log CFU/cm^2 in 16 days for GDL. Finally, the combination of the three antimicrobials seemed to control the growth of the pathogen at both storage temperatures; however, it was found to be less effective than 1.8% sodium lactate combined with 0.25% sodium diacetate. In samples containing the combination of the three antimicrobials *L. monocytogenes* populations increased by 2 log CFU/cm^2 in 70 days for product stored at 4°C and 2.1 log CFU/cm^2 in 28 days for product stored at 10°C. Bacterial growth on TSAYE and PALCAM followed similar patterns.

The data for changes in populations of other microorganisms indicated that LAB proliferated at both temperatures. GDL used singly seemed to be the treatment that controlled the growth of LAB more effectively at 10°C, but not at 4°C. At 4°C growth of LAB was inhibited by sodium lactate combined with 0.125% GDL more effectively (a 1.4 log CFU/cm^2 increase was observed in 70 days). Sodium lactate combined with 0.25% sodium diacetate, which was the most effective treatment against *L. monocytogenes*, inhibited the growth of LAB at 4°C, but not at 10°C. No major growth of yeasts and molds was observed at both storage temperatures. All treatments, except GDL (0.125%), were effective against *Pseudomonas* sp. at 4°C compared to the controls (uninoculated and inoculated controls), while at 10°C no major growth was observed. *Enterobacteriaceae* seemed to proliferate in uninoculated samples at 10°C. At 4°C the growth of *Enterobacteriaceae* was not permitted even in the untreated samples, because of the low temperature.

The pH of samples was determined in a 1:10 dilution (sample:water) with distilled water. The initial pH of control samples was 6.62±0.02. Antimicrobials used singly and sodium lactate combined with 0.125% sodium diacetate caused a pH reduction of approximately 0.1 unit on day-0. Specifically, the pH of samples that contained sodium lactate, sodium diacetate and GDL was 6.54±0.01, 6.51±0.01 and 6.54±0.02,

respectively, whereas, samples that contained sodium lactate combined with 0.125% sodium diacetate had a pH of 6.52±0.01. Sodium lactate combined with 0.125% GDL resulted in a 0.2 unit reduction (6.44±0.02). The treatments that caused a noticeable reduction in pH (day-0) were sodium lactate combined with 0.25% sodium diacetate or GDL (6.35±0.08 and 6.34±0.07, respectively) and the combination of the three antimicrobials (6.26±0.01). During storage at 4^{o}C, the samples with the lowest pH were the inoculated controls and the samples containing 0.125% GDL. At this temperature, the pH of control samples and samples that contained single GDL was 5.66±0.06 and 5.85±0.04, respectively, after storage for 35 days. At 10^{o}C, the pH of inoculated controls and samples containing single sodium diacetate or GDL was 4.81±0.01, 4.86±0.03 and 4.68±0.03, respectively, after storage for 28 days. pH measurements were also taken throughout the storage period on samples suspended in 100 ml buffered peptone water (BPW; Difco), from which microbiological analyses were performed. Control samples and samples containing single GDL had the lowest pH during storage at both temperatures, suggesting microbial growth. Specifically, the pH of control samples was reduced to 5.25 in 90 days at 4^{o}C and 5.37 in 28 days at 10^{o}C, whereas samples with 0.125% GDL in the formulation had pH of 5.52 in 90 days at 4^{o}C and 5.37 in 28 days at 10^{o}C.

The cooking yield (%) of samples varied from 90.2 (sodium lactate combined with 0.25% GDL) to 92.8 (sodium lactate combined with 0.125% GDL). The cooking yield of control samples was 90.6. The moisture content (%) of treatments varied from 53.8 to 56.8. Control samples presented the highest moisture content. The treatment with the lowest moisture content was the combination of the three antimicrobials (53.8%). This treatment also had an increased fat content (25.6%), compared to the other treatments and the control (22.8%).

Study 2: Control of *Listeria monocytogenes* with antimicrobials in the formulation and by dipping in organic acids of post-processing inoculated pork frankfurters stored at 10^{o}C in vacuum packages.

This study investigated the antimicrobial effect of additives included singly or in combination in the formulation of pork frankfurters and followed by immersion of the inoculated finished product into organic acid solutions.

The results showed that in untreated samples (control), *L. monocytogenes* populations exceeded 7.5 log CFU/cm^2 in 12 days. In samples that contained 1.8% sodium lactate or 0.25% sodium diacetate, pathogen populations exceeded 7 and 6 log CFU/cm^2, respectively, in 40 days. Sodium lactate combined with 0.25% sodium diacetate inhibited growth of the pathogen for 40 days, whereas in samples that contained sodium lactate and 0.125% sodium diacetate a 1.7 log CFU/cm^2 increase was observed in 40 days.

Dipping in LA (day-0) reduced initial populations of the pathogen by 0.7-2.1 log CFU/cm^2, but during storage (12-20 days) growth in dipped samples without antimicrobials in the formulation reached 7.9 log CFU/cm^2. Dipping in AA reduced initial populations of the pathogen by 0.7-1.7 log CFU/cm^2 but populations of the pathogen reached 5.5 log CFU/cm^2 in 20 days and 7.8 log log CFU/cm^2 in 40 days. In samples containing single antimicrobials (1.8% sodium lactate or 0.25% sodium diacetate) and dipped in LA the pathogen exceeded 2.5 log CFU/cm^2 in 28 days, whereas, in samples that contained single antimicrobials and dipped in AA, growth of the pathogen was inhibited for 28 days and reached 1.3-1.8 log CFU/cm^2 in 40 days. In samples that contained combinations of antimicrobials (1.8% sodium lactate and 0.25 or 0.125%

sodium diacetate) and dipped in LA or AA, bactericidal effects (reductions of 0.6-1.0 log CFU/cm^2 in 28-40 days were observed.

Bacterial growth on TSAYE was similar to that observed on PALCAM. LAB proliferated in untreated samples (inoculated control) where populations reached 6.8 log CFU/cm^2 in 40 days, and in samples containing 0.25% sodium diacetate where populations reached 6.7 log CFU/cm^2 in 40 days. In samples containing 0.25% sodium diacetate or sodium lactate and 0.125% sodium diacetate and dipped in LA, final populations of LAB exceeded 3.5 log CFU/cm^2 in 40 days. In samples dipped in LA, all formulation treatments seemed to allow growth, compared to the control, while the opposite was observed in samples dipped in AA. No major growth of yeasts and molds was observed throughout storage.

On day-0, pH measurements were taken of all samples in distilled water (dilution 1:10). The pH of control samples was 6.07±0.04. The formulation treatments that caused pH reductions on day-0 were 0.25% sodium diacetate (5.77±0.13) and sodium lactate combined with 0.25% sodium diacetate (5.94±0.02). The pH of samples containing 1.8% sodium lactate and sodium lactate combined with 0.125% sodium diacetate was 6.05±0.07 and 6.00±0.00, respectively. Dipping in LA reduced the pH by 0.3-0.4 units, while dipping in AA caused a reduction of 0.2-0.4 units. The initial pH of control samples (no antimicrobials in the formulation), when dipped into LA or AA, was 5.72±0.06 and 5.81±0.06, respectively, while samples containing single sodium lactate, when dipped into LA or AA had a pH of 5.64±0.01 and 5.72±0.00, respectively. The samples that contained 0.25% sodium diacetate and dipped in AA were the ones with the lowest initial pH (5.47±0.06), whereas, frankfurters with the same formulation and dipped into LA had a pH of 5.54±0.05. The initial pH of frankfurters that contained sodium lactate combined with 0.125% sodium diacetate and dipped into LA or AA was 5.58±0.01 and 5.69±0.02. Frankfurters that contained sodium lactate combined with 0.25% sodium diacetate and dipped into LA or AA had initial pH of 5.59±0.02 and 5.69±0.02, respectively. pH measurements were also performed on samples suspended in BPW.

Cooking yields (%) of frankfurters with antimicrobials in the formulation varied from 88.0 (samples containing sodium lactate combined with 0.125% sodium diacetate) to 94.6 (1.8% sodium lactate). Control samples (no antimicrobials in the formulation) had the lowest moisture content (51.9%), which resulted to a high fat content (25.2%), compared to treated frankfurters. The highest moisture content (53.1%) was observed in samples containing sodium lactate and 0.125% sodium diacetate. Fat contents of samples varied from 23.1% (samples with 0.25% sodium diacetate) to 25.2% (control samples).

Study 3: Nisaplin®, organic acids and a preservative applied as dipping solutions to control post-processing *Listeria monocytogenes* contamination of commercial bologna and ham stored at 10°C in vacuum packages.

This study evaluated the antilisterial effect of Nisaplin, acetic acid, lactic acid and potassium benzoate, applied as dipping solutions either singly or sequentially, on inoculated commercial bologna and ham.

Initial populations of *L. monocytogenes* in untreated (control) bologna and ham samples increased from 3.4 log CFU/cm^2 to 7.4 log CFU/cm^2 and 7.8 log CFU/cm^2, respectively, in 8 days at 10°C. Initial reductions of *L. monocytogenes*, compared to the controls, on bologna and ham samples treated with AA, LA and PB ranged from 0.4 to 0.7 log CFU/cm^2. Samples treated with Nisaplin showed initial reductions of 2.4 log CFU/cm^2 for bologna samples and 2.6 log CFU/cm^2 for ham samples. Similar

reductions were obtained when samples were initially immersed in Nisaplin and subsequently dipped in AA, LA and PB. In these samples, *L. monocytogenes* populations were reduced by between 2.5 and 2.8 log CFU/cm^2 for bologna samples and between 2.7 and 2.9 log CFU/cm^2 for ham samples, compared to the control samples.

Growth of *L. monocytogenes* on bologna and ham samples treated with AA, LA and PB (applied singly) was inhibited during the storage period. At the end of storage, populations of *L. monocytogenes* were reduced by 1.0, 0.9 and 0.7 log CFU/cm^2 on AA, LA and PB treated bologna samples, respectively, and 0.9, 0.7 and 1.0 log CFU/cm^2 on ham samples, respectively. In contrast to the organic acid and potassium benzoate treatments, the Nisaplin treatment, when used on its own, allowed proliferation of the pathogen during storage. In bologna samples, *L. monocytogenes* exceeded 6.5 log CFU/cm^2 in 20 days and 6.0 log CFU/cm^2 in 8 days on ham samples. Samples treated initially with Nisaplin and followed by AA showed reductions in *L. monocytogenes* populations of 1.0 and 0.7 log CFU/cm^2 in bologna and ham samples after 48 days of storage. Similarly, at the end of storage, populations on bologna and ham samples were reduced by 0.8 and 0.7 log CFU/cm^2, respectively, when samples were treated with Nisaplin followed by PB. For samples treated with Nisaplin followed by LA, *L. monocytogenes* was reduced to undetectable levels of <-0.4 log CFU/cm^2 and <-0.2 log CFU/cm^2 on bologna and ham samples, respectively, at the end of storage.

Bacterial growth on TSAYE agar followed similar patterns as that on PALCAM agar for the control samples. During the storage period, however, samples treated with AA, LA, PB and Nisaplin, applied singly, showed higher counts on TSAYE agar, reflecting growth of spoilage bacteria. Preliminary identification of the spoilage populations indicated that lactic acid bacteria predominated on AA, LA and PB treated samples, while Gram-negative bacteria predominated on Nisaplin treated samples. Samples treated with Nisaplin followed by AA, LA and PB showed no pronounced proliferation of spoilage bacteria during the 48-day storage period.

The pH of untreated bologna and ham samples on day-0 was 6.1 and 6.4, respectively. Noticeable reductions in pH were obtained for samples treated with AA and LA (reductions of 1.3 to 1.8 pH units) applied singly as well as those combined with Nisaplin (reductions of 1.4 to 2.1 pH units). Potassium benzoate and Nisaplin treatments applied on their own and in combination had no apparent effect on the product pH on day-0. These treatments, in addition to the control samples, showed changes in pH during the storage period reflecting bacterial growth of *L. monocytogenes* and/or spoilage populations. At the end of storage, control and Nisaplin (applied singly) treated samples had pH values of 4.7 and 5.1, respectively (bologna) and 4.8 and 5.2, respectively (ham).

Sensory analysis of bologna and ham treated with antimicrobials:

Sensory analysis of bologna slices treated with single treatments of AA, LA and PB indicated that the treatments had a negative effect on the odor (AA treatment only), flavor and overall acceptability ratings of the product. Flavor and acceptability scores for each of the treatments were 6.0 and 6.1, respectively (control), 4.1 and 4.3, respectively (AA), 4.9 and 4.8, respectively (LA) and 4.8 and 4.7, respectively (PB).

The same treatments also had a negative effect on the sensory qualities of ham slices. The PB seemed to affect the color and appearance of the product; scores of 3.8 and 4.7, respectively, were obtained, compared to the scores of 5.7 and 6.2, respectively, obtained for control slices. Lower odor, flavor and overall acceptability ratings, compared to the control (6.6, 6.6 and 6.2, respectively), were also obtained for

all three antimicrobial treatments. The AA treatment gave the lowest ratings for all three attributes (3.4, 2.1 and 2.5, respectively). Odor, flavor and overall acceptability ratings for the LA and PB treatments were 4.5, 3.0 and 3.2, respectively, for the LA treatment, and 4.1, 2.7 and 2.6, respectively, for the PB treatment.

Study 4: Sensory evaluation of frankfurters treated with antimicrobials.

The sensory qualities of frankfurters (formulated in our laboratory and commercial) treated with antimicrobials were evaluated in this study.

Sensory analysis of effective treatments from Study 1:

In summary, all samples had an acceptable flavor. The ratings for flavor ranged from 5.6 (control samples) to 6.6 (sodium lactate combined with 0.25% sodium diacetate). Overall acceptability ranged from 5.5 (samples containing the three antimicrobials) to 6.1 (sodium lactate combined with 0.25% sodium diacetate). Sodium lactate combined with 0.25% sodium diacetate in the formulation of frankfurters received the highest ratings for flavor and overall acceptability.

Sensory analysis of effective treatments from Study 2:

The ratings for flavor ranged from 5.5 (control samples) to 6 (sodium lactate and 0.125% sodium diacetate). Samples containing sodium lactate combined with 0.125% sodium diacetate had an overall acceptability of 6, whereas both controls and samples with sodium lactate and 0.25% sodium diacetate had an overall acceptability of 5.6.

Dipping in LA did not to seem to affect the overall acceptability of samples with no antimicrobials in the formulation. The lowest ratings for flavor (5.8) were obtained for control samples and samples containing 0.25% sodium diacetate. All dipped samples received lower ratings for odor, compared to the control samples (no antimicrobials, no dipping). Samples containing sodium lactate and 0.25% sodium diacetate and dipped in LA received the lowest ratings for odor (4.8).

Samples dipped in AA obtained similar or even higher ratings for flavor and overall acceptability, to the ones received by undipped control samples (5.5 for flavor and 5.6 for overall acceptability). The highest ratings for flavor were obtained by samples containing sodium lactate and 0.125% sodium diacetate, followed by samples with sodium lactate and 0.25% sodium diacetate (6 and 5.9, respectively). Also, dipping into AA did not seem to affect the odor scores. All treatments (except sodium lactate and 0.25% sodium diacetate), when dipped into AA received ratings for odor similar to the ones of the undipped controls (5.3). The lowest ratings for odor (4.9) were obtained for samples containing sodium lactate and 0.25% sodium diacetate and dipped in AA.

Sensory analysis of effective treatments from Study 3:

Mean ratings of the sensory attributes for the treated and untreated commercial frankfurters indicated that the different dipping treatments seemed to have no apparent effect on the sensory qualities of the product. Mean ratings for odor were similar for the control (5.2) and treated samples (5.0 to 5.7), as were the flavor acceptability scores (control, 5.8; treated samples, 5.1 to 6.0). Overall acceptability ratings for control, acetic acid, lactic acid and potassium benzoate samples were 5.8, 5.6, 6.1 and 4.9, respectively.

Study 5: Reduction of *Listeria monocytogenes* populations during exposure to a synthetic gastric fluid following storage of inoculated frankfurters formulated and treated with organic acids.

This study (not included in the original proposal) evaluated the acid tolerance response of *L. monocytogenes* on stored, inoculated frankfurters formulated with antimicrobials and/or treated with organic acids, to synthetic gastric fluid.

Survival/growth of L. monocytogenes ***on frankfurters (time-0 of exposure):***

Irrespective of antimicrobial formulations, dipping frankfurters in lactic acid (LA) or acetic acid (AA) resulted in approximately 0.5 to 1.0 log CFU/cm^2 immediate reduction as compared with nondipped (ND) franks with no major differences between samples dipped in LA and AA (time-0). As indicated previously (Study 2), growth of *L. monocytogenes* during storage in control (no antimicrobials in formulation) frankfurters was faster and greater when dipped in LA as compared to AA (time-0). As indicated, growth of *L. monocytogenes* during storage in nondipped frankfurters increased in the following order: control > sodium lactate > sodium diacetate > sodium lactate + 0.125% sodium diacetate > sodium lactate + 0.25% sodium diacetate with the last formulation (sodium lactate + 0.25% sodium diacetate) totally inhibiting growth throughout storage (time-0). Although dipping in AA totally inhibited *L. monocytogenes* growth in frankfurters irrespective of antimicrobial formulations, the pathogen grew significantly from day-20 to -40 in frankfurters formulated with sodium lactate and sodium diacetate singly but not in those samples with combined sodium lactate + sodium diacetate formulations dipped in LA (time-0).

Acid tolerance response (ATR) of L. monocytogenes ***survivors on nondipped (ND) frankfurters exposed to synthetic gastric fluid:***

At day-0, there was no survival of *L. monocytogenes* after 20 min exposure to GF although the initial stationary phase populations on the surface of the frankfurters were quite low (0.9-2.2 logs). At day-10, there was still no survival of *L. monocytogenes* on the frankfurters exposed to the GF as cells were either in exponential phase and as such very sensitive to the acid challenge or were still present in low numbers and unable to support survival. From day-20 to -40 the growth of *L. monocytogenes* in frankfurters (control, sodium lactate and sodium diacetate) increases its survival to the subsequent gastric fluid (HCl acid) stress. It is important to note that the higher the growth of the pathogen on the frankfurters (in the order: control > sodium lactate > sodium diacetate), the higher was the subsequent survival of the pathogen to the gastric challenge. Reductions of the pathogen populations after 20 min of acid challenge at day-30 were comparable (4-5 logs) in frankfurter samples with detectable survivors (control, sodium lactate and sodium diacetate) which indicates that at this time during storage, the inclusion of sodium lactate or sodium diacetate in the formulation did not result in increased acid tolerance response (ATR) and in fact at day-40, the inclusion of sodium lactate and sodium diacetate in the formulation resulted in higher (ca. 2 logs) reduction of the pathogen than the control samples. There was a major tailing effect in survival between 20 and 60 min of exposure to the GF.

ATR *of* L. monocytogenes ***survivors on LA-dipped frankfurters exposed to synthetic gastric fluid:***

Acid tolerance responses of surviving cells at days 0 and 10 in the LA-dipped samples followed a similar pattern to the ND samples. From day-20 to -40 there was a major increase in the ATR of *L. monocytogenes* in frankfurters formulated with sodium diacetate singly with the average log reductions from 0 to 20 min approximately 1-2 logs as compared with average reductions of 4-5 logs in the corresponding ND samples. The increased ATR seen from day-20 to -40 was exclusively correlated with sodium diacetate samples because sodium lactate samples at time-0 with similar initial populations did not promote survival of the pathogen. At day-20, control samples dipped in LA showed a comparable reduction in pathogen populations to ND samples, however, by day-30 there was a major increase in ATR of *L. monocytogenes* in the control samples dipped in LA (reduction approximately 2 logs), which was not maintained through day-40. The same tailing effect seen in all ND samples is repeated in the control and sodium diacetate samples dipped in LA.

ATR of L. monocytogenes ***survivors on AA-dipped frankfurters exposed to synthetic gastric fluid:***

Acid tolerance responses of surviving cells at days 0 and 10 are similar to both the ND and LA-dipped samples although the initial populations were much lower due to the acetic acid dipping. From day-20 to -40, gastric challenge survivors were only evident in control samples and showed a similar pattern to that in the control and LA-dipped samples. The increase in ATR by day-30 in the LA-dipped control samples was again evident but to a lesser extent with an approximate 3 log reduction from 0 to 20 min.

Combinations of sodium lactate/sodium diacetate in the frankfurter formulations were far more effective than the singular formulations in inhibiting *L. monocytogenes* at 10°C under vacuum storage. Sodium diacetate (0.25%) was necessary in combination with sodium lactate to completely inhibit *L. monocytogenes* growth at 10°C under vacuum storage while sodium lactate combined with sodium diacetate at 0.125% permitted growth of the pathogen. Although dipping in AA was more effective than that in LA with only control samples able to grow in the former treatment, neither was able to completely inhibit growth of *L. monocytogenes* on frankfurters at 10ºC under vacuum storage. The efficacy of dipping treatments was enhanced when frankfurters were formulated with antimicrobials especially those in combinations. The greater the growth of *L. monocytogenes* in frankfurters during storage, the higher the risk of subsequent survival in a stomach environment and correspondingly, the lower the growth potential of the pathogen in frankfurters processed with antimicrobials, the less the chance of pathogen survival in a stomach environment. Antimicrobials (sodium lactate or sodium diacetate), either singly or in combination, in the formulation of frankfurters did not appear to increase the ATR of *L. monocytogenes* survivors to synthetic gastric fluid, however, dipping treatments especially LA appeared to increase the ATR of *L. monocytogenes* to synthetic gastric fluid following growth with prolonged storage. Sodium diacetate (0.25%) included singly in the formulation of frankfurters appeared to dramatically increase the ATR of *L. monocytogenes* to synthetic gastric fluid on those frankfurters dipped in LA. Pathogen populations on frankfurters may be expected to undergo a rapid decline when exposed to synthetic gastric fluid, but after prolonged storage an acid-resistant sub-population may arise to cause infection provided the initial population is >4.0 log CFU/cm^2.

Discussion:Under the conditions of these studies, the results indicated that:
1. Combinations of antimicrobials used in the formulation of bologna or frankfurters suppressed the growth of *L. monocytogenes* more effectively than antimicrobials used singly during storage at 4°C and 10°C (Studies 1 and 2).

2. The most effective combination of antimicrobials included in the formulation of bologna (Study 1) was sodium lactate (1.8%) combined with 0.25% sodium diacetate (this treatment inhibited the growth of *L. monocytogenes* for 90 days during storage at 4°C and 28 days during storage at 10°C), followed by sodium lactate (1.8%) combined with sodium diacetate (0.125%) and GDL (0.125%).

3. The most effective combination of antimicrobials included in the formulation of frankfurters (Study 2) was sodium lactate combined with 0.25% sodium diacetate (this treatment inhibited the growth of *L. monocytogenes* for 40 days during storage at 10°C) followed by sodium lactate combined with 0.125% sodium diacetate.

4. Dipping in 2.5% AA was more effective than dipping in 2.5% LA for untreated samples and samples containing single antimicrobials (Study 2).

5. Sodium lactate (1.8%) + sodium diacetate (0.125 or 0.25%**)** in the formulation combined with dipping in 2.5% organic acid solutions resulted in *L. monocytogenes* reductions during storage at 10°C (Study 2).

6. Lower concentrations of antimicrobials in the formulation, in combination with dipping in LA or AA, can be as effective as using higher concentrations of antimicrobials in the formulation, in combination with dipping in LA or AA.

7. The dominant natural flora of bologna or frankfurters at 4°C and 10°C was LAB.

8. Dipping of commercial bologna and ham in 2.5% AA, 2.5% LA or 5% PB, applied singly or in combination with 0.5% Nisaplin, reduced pathogen levels during the 48-day storage period, at 10°C (Study 3). The combination treatments, however, were more effective in reducing *L. monocytogenes* since they caused higher initial reductions (2.5 to 2.9 log CFU/cm^2 reductions).

9. The combination treatment, Nisaplin followed by LA, reduced *L. monocytogenes* to undetectable levels in both products at the end of storage.

10. Nisaplin (0.5%), applied on its own, resulted in high initial reductions of *L. monocytogenes*, but during storage allowed proliferation of the pathogen to levels comparable to the control.

11. The sensory attributes (odor, flavor and overall acceptability) of commercial bologna and ham were negatively affected by single treatments of AA, LA and PB, as compared to control samples.

12. Sodium lactate (1.8%) combined with 0.25% sodium diacetate (the most effective formulation treatment; Studies 1 and 2) included in the formulation of frankfurters did not seem to have a negative effect on the flavor and overall acceptability of the product, when compared to the control samples.

13. Samples that contained sodium lactate (1.8%) combined with 0.125 or 0.25% sodium diacetate and dipped in LA or AA (treatments that had bactericidal effects; Study 2) received the same or higher sensory ratings than the control samples (no antimicrobials in the formulation, no dipping) for flavor and overall acceptability.

14. Dipping of commercial frankfurters in LA, AA and PB solutions had no apparent effect on their sensory qualities.

15. Under the conditions of this study, sodium diacetate (0.25%) used singly in the formulation of frankfurters appeared to increase the acid tolerance response of *L. monocytogenes* to synthetic gastric fluid when frankfurters were dipped in 2.5% lactic acid.

The results obtained under the conditions of these studies should be useful to meat processors and regulators in their efforts to control this deadly pathogen in ready-to-eat meat products. Furthermore, they could use these results as a guide to develop their own formulations to produce products that may be used to produce or formulate ready-to-eat products that are classified as 'low risk' under the FSIS Directive 10,240.3 (FSIS, 2002). Under this new directive, published on 12/9/02, a low-targeted verification program will be used by the FSIS for establishments that produce low risk products and have a science-based program addressing *L. monocytogenes* in the product, on food contact surfaces and in the environment, and make data regarding testing available to the FSIS.

Lay Interpretation: *Listeria monocytogenes*, the causative agent of listeriosis, is an important food-borne pathogen that has become a major food safety concern. This microorganism is ubiquitous in the environment and is able to withstand various cleaning and sanitation procedures; therefore, its elimination from food-processing facilities is necessary but difficult. Cooked, ready-to-eat meats have been implicated as potential sources of food-borne listeriosis. The pathogen contaminates the product mainly after thermal processing; therefore, the development of methods to control its growth during storage is necessary. The results of the studies reported here indicate that antimicrobials, such as, sodium lactate (1.8%) in combination with sodium diacetate (0.25%) can be added to the formulation of bologna and pork frankfurters in order to inhibit growth of *L. monocytogenes* during storage not only at 4°C but also at 10°C, which is considered to be an abusive temperature. Combinations of antimicrobials in the formulation followed by dipping of the product into acetic acid and lactic acid can have additional bactericidal effects on the pathogen during storage. In addition, growth of *L. monocytogenes* on commercial products, such as bologna and ham can be inhibited during storage at 10°C by dipping the products in 2.5% acetic acid, 2.5% lactic acid or 5% potassium benzoate. When these treatments are combined with 0.5% Nisaplin, higher initial reductions of the pathogen are obtained. The results of this study should be useful to the meat industry in their efforts to inactivate or inhibit the growth of this deadly pathogen on ready-to-eat meat products during storage.

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Disclaimer: The conclusions included in this report and which are derived from the results of the studies described in this report apply only to the conditions under which the studies were conducted, and do not guarantee product safety. Processors of products may consider these findings only as a measure of relative antimicrobial effects among various formulations and treatments as tested and should conduct independent testing to determine the effects of formulations/treatments on the microbiology of their products.

References:

Bedie, G.K., Samelis, J., Sofos, J.N., Belk, K.E., Scanga, J.A., and Smith, G.C. 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* post-processing contamination on frankfurters stored at 4°C in vacuum packages. J. Food Prot. 64:1949-1995.

Beumer, R.R., de Vries, J., and Rombouts, F. M. 1992. *Campylobacter jejuni* non-culturable coccoid cells. Int. J. Food Microbiol. 15:153-163.

CDC (Centers for Disease Control and Prevention) 1999. Update: Multistate outbreak of listeriosis-United States, 1998-1999. Morbid. Mortal. Weekly Rep. 47:1117-1118.

CDC 2002. Public health dispatch: Outbreak of listeriosis-Northeastern United States, 2002. Morbid. Mortal. Weekly Rep. 51:950-951.

Cotter, P.D., Emerson, N., Gahan, C. G. M., and Hill, C. 1999. Identification and disruption of *lisRK*, a genetic locus encoding a two-component signal transduction system involved in stress tolerance and virulence in *Listeria monocytogenes*. J. Bacteriol. 181:6840-6843.

Czuprynski, C.J., Faith, N. G., and Steinberg, H. 2002. Ability of the *Listeria monocytogenes* strain Scott A to cause systemic infection in mice infected by the intragastric route. Appl. Environ. Microbiol. 68:2893-2900.

Food Safety and Inspection Service (FSIS) 2002. FSIS Directive 10,240.3 – Microbial sampling of ready-to-eat (RTE) products for the FSIS verification testing program. http://www.fsis.usda.gov/oa/topics/lm.htm

Gardner, G.A. 1966. A selective medium for enumeration of *Microbacterium thermosphactum* in meat and meat products. J. Appl. Bacteriol. 29:455-460.

Lou, Y. and Yousef, A.H. 1999. Characteristics of *Listeria monocytogenes* important to food processors, p. 131-224. *In* E.T. Ryser and E.H. Marth (ed.), *Listeria*, listeriosis and food safety. Marcel Dekker Inc., New York.

Samelis, J., Sofos, J.N., Kain, M.L., Scanga, J.A., Belk, K.E. and Smith, G.C. 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4°C in vacuum packages. J. Food Prot. 64:1722-1729.

Samelis, J., Bedie, G.K., Sofos, J.N., Belk, K.E., Scanga, J.A., and Smith, G.C. 2002.

Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4°C in vaccum packages. J. Food Prot. 65:299-307.

Tompkin, R.B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. J. Food Prot. 65:709-725.

Tompkin, R.B., Scott, V.N., Bernard, D.T., Sveum, W.H. and Gombas, S. 1999. Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. Dairy Food Environ. Sanit. 19:551-562.