Evaluation Of Antimicrobials Incorporated into the Formulation Against Post-Processing Contamination of *Listeria monocytogenes* on Frankfurters Stored at 4°c in Vacuum Packages

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

SUMMARY

Listeria monocytogenes has become a major concern for the meat processing industry worldwide, creating a need for control of post-processing contamination in cured meat products. Research on the addition of lactates, acetates and other chemicals to the formulation of cured meat products has been intensified with good potential. The goal of this project was to find effective combinations of antimicrobials for the control of *L. monocytogenes* introduced in frankfurters post-processing.

Key Words: frankfurter, antimicrobials, *Listeria monocytogenes*

INTRODUCTION

Listeria monocytogenes is ubiquitous in nature and may be developing resistance to food preservation methods (Lou and Yousef, 1999). It has the ability to colonize in meat plants (Samelis and Metaxopoulos, 1999), and may survive under unfavorable conditions in meat environments (Farber and Peterkin, 1999; Harmayani *et al*., 1993; Samelis and Metaxopoulos, 1999; Tompkin *et al*., 1999). Unfortunately, sanitation strategies and hygienic practices applied in the meat industry are often insufficient to prevent recontamination and growth of *L. monocytogenes* in processed meats, where the pathogen is transferred mainly post-cooking (Farber and Peterkin, 1999; Samelis and Metaxopoulos, 1999; Tompkin *et al*., 1999). Thus, there is a need for evaluation of the effects of antimicrobials, added singly or in combinations in the formulation of cured meats (Wederquist *et al*., 1994), against post-processing contamination of *L. monocytogenes* on frankfurters.

MATERIALS AND METHODS

Preparation of pork frankfurters. Fresh pork trimmings of approximately 30% fat were obtained from Swift Company (Greeley, CO). Frankfurter 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). Each experimental batch of frankfurters was divided in equal portions, and the appropriate amount (% by weight of total formulation) of each of the antimicrobials was mixed with the other ingredients in the chopper. The final temperature of the frankfurter batter after mixing was 5°C. The batter was extruded into 24 mm diameter, fibrous cellulose casings (Koch, Kansas City, MO) and weighed. The frankfurters were cooked in a smokehouse (Alkar, Lodi, WI) to a final core temperature of 70°C (155°F). After cooking, the frankfurters were showered for 5 min with cool tap water and stored at 4°C overnight. The next day, the casings were removed manually and frankfurters (11 cm length) were obtained, yielding an external surface of 83 cm^2. Peeled frankfurters were wrapped in meat wrapping paper and transferred to the microbiology laboratory for inoculation, vacuum packaging, storage and analyses.

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 10^3-10^4 CFU/cm^2 of inoculated product. This composite inoculum was used to inoculate the frankfurters. 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. Two frankfurters were aseptically transferred to vacuum bags (20 x 25 cm, 3 mil std barrier, Nylon/PE vacuum pouch; Koch, Kansas City, MO). The composite inoculum (0.25 ml) was deposited on each frankfurter and spread over the product surface by swirling the sample by hand from the outside of the bag. The target inoculation level of frankfurters to evaluate antimicrobials in the formulation, singly or combined, was 10^3-10^4 CFU/cm^2.

Treatments of frankfurters with antimicrobials and micro-biological analysis. Frankfurters were prepared with single antimicrobials in the formulation: sodium acetate (0.25% or 0.5%), sodium diacetate (0.25% or 0.5%) and sodium lactate (3% or 6%, calculated as pure compound of a 60% commercial product). The amounts added to the batter included those that were recently announced as permissible levels by FSIS (2000) to be used as antimicrobials or flavoring agents in meat products (FSIS, 2000). The potential to increase sodium acetate and sodium diacetate from 0.25% to 0.5%, as suggested by FSIS, and sodium lactate from 3% to 6% was also evaluated. Antimicrobials combined in the formulation such as sodium lactate (3%) plus sodium acetate (0.25%), sodium lactate (3%) plus sodium diacetate (0.25%), and sodium lactate (3%) plus glucono-D-lactone (GDL, 0.25%) were also evaluated.

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 homogenized in a stomacher (Masticator, IUL

Instruments, Barcelona, Spain) for 30 sec. 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 0.8 log CFU/cm^2, 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 frankfurter surface (166 cm^2) to result in 6 CFU/cm^2, i.e. 0.8 log CFU/cm^2. 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

In stored (4°C) frankfurters without antimicrobials in the formulation, *L. monocytogenes* increased by 1-2 logs after 10 days, and exceeded 10^8 CFU/cm^2 with prolonged storage (Table 1). Pathogen growth was inhibited for approximately 50 days at 4°C when 3% sodium lactate was added to the formulation (Table 2), while its effect was bacteriostatic, or even bacteriocidal, and lasted for 90 days when the concentration was increased to 6% (Table 1). In contrast, sodium acetate failed to inhibit growth of *L. monocytogenes* for more than 20-35 days at either concentration (0.25% or 0.5%); however, the growth rate of the pathogen was retarded by 1-2 logs (from day 20 to day 90) in frankfurters prepared with 0.5% compared to 0.25% sodium acetate (Table 1). *Listeria monocytogenes* was able to grow in the presence of 0.25% sodium diacetate in the formulation after 35-50 days at 4°C and eventually increased to average levels of 10^6 CFU/cm^2. However, frankfurters prepared with 0.5% sodium diacetate demonstrated a bacteriostatic or even a slight bacteriocidal effect on *L. monocytogenes* throughout storage (90 days) (Table 1).

Various combinations of antimicrobials in the formulation of frankfurters were used in attempts to increase control of growth of *L. monocytogenes*. Results showed that combinations of antimicrobials were more effective than their single antimicrobial counterparts used at the same concentrations. Sodium lactate (3%) did not inhibit growth of *L. monocytogenes* for more than 50 days at 4°C, but the combination of 3% sodium lactate with 0.25% of sodium acetate, sodium diacetate or GDL, demonstrated a bacteriostatic effect at 90 days (Table 2). This trend was also observed in the changes of total bacterial counts (data not shown).

Sodium diacetate (0.25 and 5%) was the only antimicrobial that reduced the pH of the frankfurters (day 0). However, during storage at 4°C, pH values increased, especially in samples with 0.25% sodium diacetate, due to the buffering capacity of the meat and because the populations of *L. monocytogenes* were maintained below 10^7 CFU/cm^2, a level above which the metabolic activity of pathogenic bacteria could lower the product pH (data not shown).

IMPLICATIONS

Listeria monocytogenes was able to multiply to a 10^6-10^8 CFU/cm^2 level during refrigerated storage (4°C) without the addition of antimicrobials in the formulation indicating a need to incorporate antimicrobials in the cured meat formulation to increase product safety and consumer confidence. The most promising antimicrobials for controlling growth of *L. monocytogenes* in frankfurters, with potential use in the meat industry, were sodium diacetate (0.5%) and sodium lactate (6%) when applied singly. The antilisterial effect of 3% sodium lactate was increased in magnitude and duration when it was combined with 0.25% of sodium acetate, sodium diacetate or GDL in the formulation, confirming that antimicrobials in multi-hurdle form have a greater portential to control *L. monocytogenes*. Based on these findings, because sodium lactate (3%) and sodium acetate/diacetate (0.25%) applied singly were not effective, it might be more efficacious to use sodium lactate and sodium acetate/diacetate in combination rather than using them individually at the new higher levels considered permissible by USDA. Any antimicrobial compound has the potential to alter the sensory characteristics of the product indicating a need to conduct product development and sensory quality studies before commercial application.

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Table 1. Changes (log cfu/cm^2 ± SD; n=9) in populations of inoculated *L. monocytogenes* (PALCAM agar) on frankfurters with or without antimicrobials in the formulation, vacuum packaged and stored at 4^{o}C.

Treatments	Days of storage at 4^{o}C						
	0	10	20	35	50	70	90
Uninoculated: No treatment	<0.8	<0.8	0.9 (0.1)	0.9 (0.1)	1.1 (0.4)	1.6 (0.7)	<0.8
Inoculated: No treatment	3.2 (0.4)	4.9 (1.2)	6.2 (1.2)	7.8 (0.6)	8.2 (0.4)	8.3 (0.2)	8.2 (0.1)
Sodium acetate (0.25%)	3.3 (0.7)	3.0 (0.5)	3.8 (0.9)	5.2 (1.7)	6.7 (2.3)	7.1 (2.0)	7.5 (0.9)
Sodium acetate (0.50%)	3.3 (0.6)	2.6 (0.1)	3.2 (0.6)	4.2 (1.4)	4.9 (2.3)	5.8 (2.9)	5.8 (2.3)
Sodium diacetate (0.25%)	3.2 (0.5)	2.5 (0.1)	3.3 (1.6)	3.7 (1.2)	4.4 (1.9)	5.2 (3.0)	5.6 (3.1)
Sodium diacetate (0.50%)	3.4 (0.7)	2.4 (0.0)	2.9 (0.5)	2.6 (0.6)	2.2 (0.7)	1.9 (0.9)	1.5 (0.7)
Sodium lactate (3%)	3.2 (0.6)	2.4 (0.1)	2.8 (0.6)	2.9 (0.7)	2.7 (0.8)	3.9 (1.5)	4.9 (2.1)
Sodium lactate (6%)	3.2 (0.7)	2.4 (0.1)	2.7 (0.6)	2.7 (0.8)	2.6 (0.7)	2.4 (0.8)	1.9 (1.2)

Table 2. Changes (log cfu/cm^2 ± SD; n=3) in populations of inoculated *L. monocytogenes* (PALCAM agar) on frankfurters with or without antimicrobials in the formulation, vacuum packaged and stored at 4^{o}C.

Treatments	Days of storage at 4^{o}C						
	0	10	20	35	50	70	90
Uninoculated: No treatment	<0.8	<0.8	1.2 (0.5)	0.8 (0.1)	1.2 (0.9)	1.7 (0.9)	1.8 (0.0)
Inoculated: No treatment	3.0 (0.0)	2.6 (0.1)	4.8 (0.3)	6.4 (0.2)	6.4 (0.7)	7.9 (0.2)	7.5 (0.1)
Sodium lactate (3%)	3.0 (0.0)	2.5 (0.1)	2.6 (0.3)	1.6 (0.5)	4.6 (0.4)	6.5 (0.3)	5.3 (0.5)
Sodium lactate (3%) plus sodium acetate (0.25%)	3.0 (0.1)	2.6 (0.1)	2.5 (0.1)	1.9 (0.2)	2.1 (0.1)	2.3 (0.1)	2.1 (0.1)
Sodium lactate (3%) plus sodium diacetate (0.25%)	3.0 (0.0)	2.5 (0.1)	2.6 (0.1)	1.9 (0.2)	2.1 (0.1)	2.3 (0.0)	2.2 (0.1)
Sodium lactate (3%) plus GDL (0.25%)	3.8 (0.4)	2.5 (0.1)	2.5 (0.1)	1.7 (0.4)	2.0 (0.2)	2.3 (0.1)	2.2 (0.1)

NA: not analyzed