SCREENING OF ANTIMICROBIALS AGAINST *LISTERIA MONOCYTOGENES* IN PORK BOLOGNA

John Samelis, Katri Strohecker, John N. Sofos, Keith E. Belk. John A. Scanga and Gary C. Smith

Center for Red Meat Safety, Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523-1171, USA

Background

Listeria monocytogenes has become a concern to the meat industry worldwide. The organism is ubiquitous, so processing and hygienic practices are often insufficient to assure zero incidence or prevent growth in processed meats (Samelis and Metaxopoulos, 1999), to which the pathogen is transferred mainly post-cooking. Because of this, a need exists to identify post-packaging "hurdle" technologies to inactivate or restrain growth of *L. monocytogenes* in meat products. Since emerging technologies, such as irradiation, are not approved, increasing interest in the incorporation of chemical (e.g., lactates, acetates, sorbates) or biological (e.g., bacteriocins) antimicrobial compounds as a safety barrier has been renewed (El-Khateib *et al.*, 1993; Wederquist *et al.*, 1994; Blom *et al.*, 1997).

Objectives

The objective of this study was to screen concentrations of various antimicrobials in dipping solutions for their potential to inactivate or control growth of *L. monocytogenes* inoculated after processing on the surface of pork bologna slices. The slices were either inoculated prior to dipping, or dipped prior to inoculation, vacuum packaged and stored at 4°C.

Methods

Pork bologna (65 mm in diameter) was prepared using raw pork trimmings (40% fat) and standard commercial practices. Sliced bologna was surface-inoculated with 10^3-10^4 cells/cm^2 of ten *L. monocytogenes* strains: Scott A (human isolate), four pork sausage isolates, one pork meat isolate, and four pork variety meat isolates. The inoculated slices were let stand separately at 5°C for 15 min, to allow for inoculum attachment. Inoculation was done before and, in some cases, after dipping for 2 min in filter-sterilized solutions (%, w/v) of acetic acid (Fisher Scientific, St.Louis, MO), lactic acid (85% w/w, Purac Inc., Lincolnshire, IL), sodium acetate (Sigma, St. Louis, MO) and sodium diacetate (Niacet, Niagara Falls, NY) with or without nerolidol (Sigma), potassium benzoate (Sigma), potassium sorbate (Sigma) and nisin (Nisaplin, Aplin & Barrett Ltd). Single slices were aseptically transferred into 6 x 8.5 cm vacuum bags (Koch, Kansas City, MO) and vacuum packaged. Uninoculated and inoculated slices dipped in sterile distilled water as well as inoculated slices without any treatment served as controls. Duplicate samples were analyzed periodically (4°C) by spread-plating in duplicate on tryptic soy agar with 0.6% yeast extract (TSAYE) and PALCAM (Difco) agar plates. Counts were expressed as log CFU/cm^2 of bologna after 48 h at 35°C. Based on slice surface (33 cm^2), the lowest detection limit of the analysis was 0.9 log CFU/cm^2. Also the pH of each sample was determined after samples were plated.

Results and Discussion

Effects of treatment of sliced bologna on *L. monocytogenes* growth are presented in Tables 1-4. The pathogen increased to as high as 10^7-10^9 cfu/cm^2 during storage at 4°C in inoculated controls. Its growth was always greater in the control samples dipped in water than in the control samples with no treatment. This indicated that dipping in water enhanced growth of *L. monocytogenes*, probably by increasing water activity on the interface of the meat surface and the packaging film. Changes of inoculated *L. monocytogenes* on PALCAM plates were very similar to those of total bacterial counts on TSAYE (results not shown), indicating that the increases of the natural flora during storage did not interfere with *L. monocytogenes* growth. These results confirmed that *L. monocytogenes* is a concern to the meat industry because it grows in refrigerated processed meats.

Lactic acid (11.5% in dipping solution), acetic acid (5.5%) and sodium diacetate (13%) were, in decreasing order, the most effective antimicrobials for inactivating *L. monocytogenes* in bologna (Tables 1-2). By reducing, however, the acid concentration to 6% and 3% for lactic and acetic acid, respectively, a bacteriostatic rather than bactericidal effect was noted (data not shown). Interestingly, both acids seemed to be more effective when slices were inoculated and then dipped rather than dipped and then inoculated (Table 1). Sodium diacetate (13%) was less effective than organic acids, while the addition of increasing concentrations (0.5 to 5.0 mM) of nerolidol to sodium diacetate did not increase its bactericidal effect (Table 2). Sodium acetate (8%) could control growth of, but not inactivate, *L. monocytogenes*; in this case the inoculation-dipping sequence had no significant effect (Table 1). Potassium sorbate and potassium benzoate prevented *L. monocytogenes* growth when at concentrations of 3-6%, but not at 1% (Table 3). In summary, higher concentrations of sodium acetate (0.25-0.5%) and potassium sorbate (0.26%) than those reported to be effective when added directly in the formulation (Harmayani *et al.*, 1993; Wederquist *et al.*, 1994; Blom *et al.*, 1997) are required in dipping solutions to control *L. monocytogenes* in processed meats.

Nisin (4000-5000 IU/ml) failed to control *L. monocytogenes* in sliced bologna (Table 4 and data not shown). In fact, nisin killed and/or injured many cells immediately after dipping, but the survivors were able to recover and grow after 1-2 weeks at 4°C. These results are in agreement with the limited antilisterial activity of nisin reported for fresh meat (El-Khateib *et al.*, 1993). Interestingly though, when slices of bologna were first immersed in nisin (4,000 IU/ml) and then inoculated, the bacteriocin exerted a strong bacteriostatic effect for up to 10 days, unlike that evidenced when the sequence of treatment was reversed (Table 4). Probably, when contamination occurs prior to treatment with nisin, cells are protected by attachment to or entrapment in the rough meat-product surface. Thus, dipping or spraying with nisin immediately after removal of casings may improve safety or processed meats safety during post-handling operations in industrial practice.

Table 1. Changes (log CFU/cm^2) in populations of inoculated *L. monocytogenes* (PALCAM agar) on bologna slices immersed for 2 min in lactic acid, acetic acid or sodium acetate decontamination solutions, vacuum packaged and stored at 4°C

Treatments	Days of storage at 4°C							
	0	4	8	11	18	26	34	41
Inoculation/No treatment	4.3	4.4	4.3	4.2	4.6	6.2	7.4	7.4
Inoculation/Water immersion	5.4	4.6	4.5	4.5	5.9	6.9	8.5	8.1
Inoculation/Acetic acid (5.5%) immersion	4.1	3.8	3.8	3.5	2.5	3.4	<0.9	<0.9
Acetic acid (5.5%) immersion/Inoculation	4.4	3.6	4.0	3.9	3.8	3.7	4.3	3.6
Inoculation/Lactic acid (11.5%) immersion	3.5	2.6	2.6	2.2	<0.9	<0.9	<0.9	<0.9
Lactic acid (11.5%) immersion/Inoculation	3.8	2.4	3.3	3.1	2.6	2.7	1.1	1.3
Inoculation/Sodium acetate (8%) immersion	4.2	4.2	4.2	2.6	4.1	4.1	4.0	3.9
Sodium acetate (8%) immersion/Inoculation	4.5	4.4	4.4	3.4	4.2	4.1	4.0	3.9

Table 2. Changes (log CFU/cm^2) in populations of inoculated *L. monocytogenes* (PALCAM agar) on bologna slices immersed for 2 min in sodium diacetate decontamination solutions supplemented with nerolidol, vacuum packaged and stored at 4°C

Treatments	Days of storage at 4°C								
	0	4	8	11	16	22	29	37	44
Inoculation/No treatment	3.9	3.5	3.6	3.9	4.4	5.3	6.6	6.6	7.8
Inoculation/Water immersion	3.7	3.4	4.0	4.8	5.7	7.3	7.9	8.3	8.1
Inoculation/Sodium diacetate (13%) immersion	3.6	3.4	3.2	3.2	3.0	1.8	2.6	2.5	2.1
Inoculation/Sodium diacetate (13%) plus 0.5 mM nerolidol immersion	3.5	3.3	3.3	3.2	3.2	1.9	2.6	2.7	2.2
Inoculation/Sodium diacetate (13%) plus 1.0 mM nerolidol immersion	3.5	3.5	3.4	3.2	3.0	1.4	2.4	2.4	2.0
Inoculation/Sodium diacetate (13%) plus 5.0 mM nerolidol immersion	3.6	3.4	3.2	3.2	3.0	2.7	2.7	2.6	2.3

The bologna pH after manufacture was 6.4-6.5. Dipping in acetic acid, lactic acid or sodium diacetate caused an immediate reduction of the product pH to 5.1-5.4, 4.8-5.0 and 5.2, respectively. Following that, the pH remained constant or increased 0.2-0.5 units during storage at the lower acid concentration. In contrast, potassium benzoate, potassium sorbate and sodium acetate caused no significant changes in bologna pH. In the control samples and in those where antimicrobials failed to restrain growth of *L. monocytogenes* below 10^7-10^8 cfu/cm^2, it was the pathogen that caused a remarkable drop in pH (to 5.5-5.8) by late storage.

Conclusions

Listeria monocytogenes may be controlled in pork products by post-processing decontamination solutions of lactic, acetic, sorbic and benzoic acids and their salts. However, the more effective antimicrobials also reduced product pH. Thus, the concentrations needed should be refined and their influence on product quality should be evaluated. Antimicrobials that showed little or no inhibitory effect, such as nisin, may prove effective if used in combinations or at higher concentrations.

Table 3. Changes (log CFU/cm^2) in populations of inoculated *L. monocytogenes* (PALCAM agar) on bologna slices immersed for 2 min in potassium benzoate or potassium sorbate decontamination solutions, vacuum packaged and stored at 4°C

Treatments	Days of storage at 4°C						
	0	7	10	20	35	50	70
Inoculation/No treatment	4.6	5.1	6.0	7.2	7.0	8.9	7.8
Inoculation/Water immersion	4.3	5.5	6.3	7.9	8.1	9.2	7.3
Inoculation/Potassium benzoate (1%) immersion	4.2	4.4	4.6	5.5	6.5	8.6	7.2
Inoculation/Potassium benzoate (3%) immersion	4.3	4.4	4.4	4.3	3.4	4.5	3.2
Inoculation/Potassium benzoate (6%) immersion	5.1	4.9	5.1	5.2	4.4	4.1	3.2
Inoculation/Potassium sorbate (1%) immersion	4.3	4.2	4.5	5.8	6.9	8.5	7.6
Inoculation/Potassium sorbate (3%) immersion	4.8	5.2	5.3	4.5	5.1	5.6	5.4
Inoculation/Potassium sorbate (6%) immersion	4.2	3.9	4.1	4.0	3.3	4.4	3.7

Table 4. Changes (log CFU/cm^2) in populations of inoculated *L. monocytogenes* (PALCAM agar) on bologna slices immersed for 2 min in nisin decontamination solutions, vacuum packaged and stored at 4°C

Treatments	Days of storage at 4°C						
	0	7	10	20	35	50	70
Inoculation/No treatment	4.6	5.1	6.0	7.2	7.0	8.9	7.8
Inoculation/Water immersion	4.3	5.5	6.3	7.9	8.1	9.2	7.3
Inoculation/Nisin (4000 IU/ml) immersion	3.0	5.7	5.8	7.5	8.1	8.8	7.6
Nisin (4000 IU/ml) immersion/Inoculation	2.7	2.4	2.7	5.3	6.9	9.0	7.8

Pertinent Literature

Blom, H., E. Nerbrink, R. Dainty, T. Hagtvedt, E. Borch, H. Nissen, and T. Nesbakken. 1997. Int. J. Food Microbiol. 38:71-76.
El-Khateib, T., A.E. Yousef and H.W. Ockerman. 1993. J. Food Prot. 56: 29-33.
Harmayani, E., J.N. Sofos, and G.R. Schmidt. 1993. Int. J. Food Microbiol. 18: 223-232.
Samelis, J. and J. Metaxopoulos. 1999. Food Microbiol. 16: 465-477.
Wederquist, H.J., J.N. Sofos and G.R. Schmidt. 1994. J. Food Sci. 59: 498-500, 516.