

Inhibition of *Listeria monocytogenes* by Sodium Diacetate and Sodium Lactate on Wieners and Cooked Bratwurst

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ABSTRACT

The inhibition of *Listeria monocytogenes* by sodium lactate and sodium diacetate was evaluated for wieners containing pork, turkey, and beef and for cooked bratwurst containing beef and pork. Both products were supplied by commercial manufacturers. Treated products were surface-inoculated with 10⁵ CFU of *L. monocytogenes* per package and vacuum-packed in gas-impermeable pouches. Wieners were stored for 60 days at 4.5°C, and bratwurst were stored for 84 days at 3 and 7°C. A surface treatment that consisted of dipping wieners into solutions containing ≤6% lactate and ≤3% diacetate for 5 s did not delay pathogen growth compared with that for untreated wieners. In additional trials, the antilisterial activity of lactate and diacetate in wiener and bratwurst formulations was evaluated. Lactate levels ranged from 1.32 to 3.4%, and diacetate was evaluated at 0.1 and 0.25%. The growth of *L. monocytogenes* was delayed for 4 and 12 weeks at 7 and 3°C, respectively, on uncured, unsmoked bratwurst formulated with 3.4% lactate/0.1% diacetate, compared with 1 and 2 weeks, respectively, for the formulation containing 2% lactate. *L. monocytogenes* grew by ≥1 log unit after 4 weeks' storage at 3 or 7°C on cured, smoked bratwurst without lactate or diacetate, but growth was inhibited for 12 weeks on cured, smoked bratwurst formulated with 3.4% lactate and 0.1% diacetate. Sodium lactate levels of ≥3% and combinations of ≥1% lactate plus ≥0.1% diacetate prevented listerial growth on wieners stored for 60 days at 4.5°C. These results indicate that dipping wieners in lactate-diacetate solutions is not an efficient way to apply these antimicrobial agents to wieners. However, the inclusion of combinations of sodium lactate and sodium diacetate in wiener or bratwurst formulations inhibits the growth of *L. monocytogenes* at ≤7°C, and an additional margin of safety was observed for products that are cured and smoked.

During the 1980s and the early 1990s, sporadic cases and several outbreaks of listeriosis in the United States and various other countries were associated with the consumption of contaminated ready-to-eat (RTE) meats (20, 29, 36). More recently, hot dogs and deli meats were implicated in multistate outbreaks in 1998 to 1999 and in the latter half of 2000 that resulted in 101 and 29 cases of listeriosis, respectively (1, 2, 8). After the 1998-to-1999 outbreak, the U.S. Department of Agriculture's Food Safety and Inspection Service increased its surveillance for *Listeria monocytogenes* in RTE meat and poultry products, resulting in a 10-fold increase in recalls in 2000 as compared with the number of recalls in 1997 (16).

The growth of *L. monocytogenes* in RTE meats has previously been described (4, 17, 27). Uncured products such as sliced poultry products support the most rapid growth. Retail wieners vary considerably in their ability to support listerial growth depending on the growth of competitive lactic acid bacteria, pH reduction, and the degree to which inhibitory smoke components are present (6, 12, 17, 22, 35).

The draft risk assessment for *L. monocytogenes* in RTE foods ranks products that support moderate to high growth

rates as posing a high risk of illness for a susceptible population (14, 15). According to the assessment, luncheon meats sliced at delicatessens support a high rate of listerial growth during storage, but their relatively short shelf life decreases their risk. Frankfurters, having a moderate risk of postprocessing contamination and growth of *L. monocytogenes* during their extended shelf life, are considered a relatively low risk product if they are properly reheated. However, if they are not reheated, contaminated frankfurters are considered a high-risk product and are ranked with pâté and meat spreads as first or second in relative risk among RTE foods. The growth of *L. monocytogenes* on frankfurters may also increase the risk of cross-contamination in a consumer's refrigerator with foods that may not be heated prior to consumption (15, 35). The risk assessment document suggests that the goal of food manufacturers should be to reduce the growth rate of *L. monocytogenes* in order to reduce the risk to the consumer (15).

Postpackaging intervention strategies (e.g., irradiation, high-pressure pasteurization, and heat pasteurization) are being developed to inactivate the pathogen. In the interim, the industry must rely on stringent environmental control of *L. monocytogenes* (34) in conjunction with product formulations that include compounds exhibiting listeristatic or listericidal properties.

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The U.S. Department of Agriculture's Food Safety and Inspection Service recently issued a final rule that increased the permissible levels of sodium lactate (SL) and potassium lactate to 4.8% and increased that of sodium diacetate (SD) to 0.25% in meat and poultry products to inhibit the growth of *L. monocytogenes* and *Clostridium botulinum* (13). Previous research has suggested that combinations of diacetate and lactate may inhibit the growth of *L. monocytogenes* in turkey slurries stored at refrigeration temperatures (28).

In this study, we evaluated the antimicrobial agents lactate and diacetate in commercially prepared products. Separate trials were undertaken to determine the efficacy of SL and SD in inhibiting surface growth of *L. monocytogenes* on wieners and cooked bratwurst. The antilisterial activity of SD and SL was first evaluated for a surface treatment consisting of dipping wieners in solutions. In subsequent trials, we evaluated the efficacy of the antimicrobial agents as part of the formulations of wieners and cooked bratwurst.

MATERIALS AND METHODS

Preparation of bacterial inocula and confirmation of *L. monocytogenes*. *L. monocytogenes* strains Scott A (clinical isolate, serotype 4b), LM 101 (hard salami isolate, 4b), LM 108 (hard salami isolate, 1/2a), LM 310 (goat's milk cheese isolate, 4), and V7 (raw milk isolate, 4) were grown individually in 10 ml Trypticase soy broth (BBL, Cockeysville, Md.) at 37°C for 16 to 18 h. Cells were harvested by centrifugation (2,500 × g, 20 min) and suspended in 4.5 ml of 0.01 M phosphate-buffered saline (pH 7.2). Equivalent numbers of each isolate were combined to provide a five-strain mixture of *L. monocytogenes* for inoculation of products. Populations of each strain and of the mixture were verified by plating on Trypticase soy agar and modified Oxford agar (21) (*Listeria* selective agar base, Oxford formulation; Oxoid Ltd., Basingstoke, Hampshire, UK). The target inoculum level was 10⁵ CFU per package.

Preparation, inoculation, and sampling of meat products: proximate analyses. Proximate analyses of moisture, protein, fat, salt, and residual nitrite were performed by the manufacturers on uninoculated samples before the samples were transported to the

Food Research Institute (Table 1). Residual sodium nitrite levels in wieners were verified by the Food Research Institute by method 973.31 of the Association of Official Analytical Chemists (23).

Preparation, inoculation, and sampling of meat products: lactate plus diacetate as a surface treatment for wieners. Fully cooked, naturally smoked wieners were obtained from a commercial producer for evaluation. The ingredients of these wieners included pork, turkey, water, beef, salt, corn syrup, flavorings, dextrose, sodium ascorbate, sodium nitrite, and extract of paprika. Precook sodium nitrite levels of 156 ppm and residual nitrite levels of <2 ppm after cooking and chilling were reported by the manufacturer.

For one trial, wieners were placed in sterile polypropylene baskets, immersed in the antimicrobial test solution, agitated for 2 min, and allowed to drain for 15 min. A second trial was performed to determine whether the lactate-diacetate solutions were effective after a shorter exposure time. In the second trial, wieners were immersed in test solutions, agitated for 5 s, and drained for 10 s.

For trial 1, wieners were (i) not treated (control), (ii) dipped in solution containing 3% SD, (iii) dipped in solution containing 6% SL, and (iv) dipped in solution containing 6% SL and 3% SD. The concentrations chosen were the maximum levels that were organoleptically acceptable for a commercial manufacturer. For trial 2, wieners were (i) not treated (control), (ii) dipped in solution containing 6% SL and 3% SD, and (iii) dipped in solution containing 3% SL and 1.5% SD. Solutions were prepared fresh with SL (wt/vol; 60% aqueous solution, calculated on an anhydrous basis; Wilke International, Inc., Lenexa, Kans.) and SD (wt/vol; powder; Mak Wood, Inc., Thiensville, Wis.) in cold (~5°C), sterile, deionized water and were used within 1 h. At 20.8°C, the final pH values were 4.7 for 3% SD, 6.2 for 6% SL, and 4.9 for SL-SD solutions.

Wieners were repackaged in gas-impermeable pouches (Clear-Tite 51/52, nylon/polyethylene [O₂ transmission of 1.2 cm³/645 cm² per 24 h at 23°C and 0% relative humidity; H₂O transmission of 0.4 g/645 cm² per 24 h at 37°C and 100% relative humidity]; Curwood, Oshkosh, Wis.), with sterile gloves being used in the creation of sampling units of 10 links each (454 g per package). The product was then surface-inoculated with 0.2 ml of *L. monocytogenes* mixture on multiple areas of the surface of each

TABLE 1. Proximate analysis^a of processed meat products before inoculation with *L. monocytogenes*

Product	Lactate (%)	Diacetate (%)	Moisture (%)	Protein (%)	Fat (%)	Salt (%)	pH	Residual nitrite (ppm)
Wiener, trial 1	— ^b	—	52.8	10.3	30.2	2.3	6.3	2
Wiener, trial 2-1	1.32	—	54.9	10.5	27.6	1.9	6.4	28
Wiener, trial 2-2	2.0	—	54.6	10.3	27.8	1.6	6.2	10
Wiener, trial 2-3	2.5	—	54.3	10.1	26.7	1.8	6.3	19
Wiener, trial 2-4	3.0	—	53.8	10.3	27.8	1.9	6.3	31
Wiener, trial 2-5	3.5	—	54.3	12.2	29.5	1.9	6.3	14
Wiener, trial 2-6	1.0	0.1	54.4	10.4	27.7	2.1	6.1	4
Wiener, trial 2-7	1.0	0.25	55.6	10.2	27.7	1.9	5.9	<2
Wiener, trial 2-8	2.0	0.1	54.6	10.0	28.4	1.9	6.2	7
Bratwurst, smoked 1	—	—	55.2	11.3	29.3	2.0	6.1	NT ^c
Bratwurst, smoked 2	3.4	0.1	53.2	11.3	29.6	1.9	6.0	NT
Bratwurst, unsmoked 1	2.0	—	51.5	12.7	31.4	1.6	6.1	NT
Bratwurst, unsmoked 2	3.4	0.1	50.9	11.8	33.1	1.5	6.1	NT

^a Provided by the manufacturers.

^b —, none added.

^c NT, not tested.

TABLE 2. Changes in populations of *L. monocytogenes* on wieners surface-treated with sodium diacetate (SD) or sodium lactate (SL) and stored at 4.5°C^a

	Day						
	0	1	7	14	30	45	60
Trial 1A^b							
NT ^c	5.86 ± 0.02	5.77 ± 0.04	— ^d	—	7.59 ± 0.02	7.74 ± 0.03	—
3% SD	5.75 ± 0.08	5.72 ± 0.08	—	—	5.69 ± 0.11	6.33 ± 0.03	—
6% SL	5.88 ± 0.07	5.72 ± 0.04	—	—	7.03 ± 0.09	7.60 ± 0.03	—
3% SD + 6% SL	5.96 ± 0.05	5.93 ± 0.08	—	—	5.93 ± 0.17	5.66 ± 0.09	—
Trial 1B^e							
NT	4.71 ± 0.06	—	4.67 ± 0.11	4.73 ± 0.05	4.62 ± 0.07	4.89 ± 0.38	5.42 ± 0.71
3% SD + 6% SL	4.61 ± 0.03	—	4.62 ± 0.05	4.60 ± 0.08	5.28 ± 1.08	4.89 ± 0.25	5.93 ± 0.36
1.5% SD + 3% SL	4.79 ± 0.06	—	4.72 ± 0.01	4.80 ± 0.12	4.64 ± 0.09	4.98 ± 0.57	5.22 ± 0.42

^a Shown are average population numbers (log₁₀ CFU per package) ± standard deviation for triplicate packages.

^b Wieners were immersed in solution, agitated for 2 min, and drained for 15 min before inoculation.

^c NT, no surface treatment.

^d —, not tested.

^e Wieners were immersed in solution, agitated for 5 s, and drained for 10 s before inoculation.

link with a pipette tip to yield a concentration of 10⁵ CFU per package. Control samples of each treatment were similarly re-packaged without inoculation. Packages were vacuum-sealed with a MultiVac AGW vacuum packager (Sepp Haggemuller KG, Wolfertschwenden, Germany) and stored at 4.5°C for up to 60 days.

Preparation, inoculation, and sampling of meat products: lactate and diacetate in wiener formulations. Wiener formulations were prepared by a commercial manufacturer. Ingredient lists were the same as those for the surface treatment trials described above, but included 1.32, 2.0, 2.5, 3.0, or 3.5% SL or 1.0% SL/0.1% SD, 1% SL/0.25% SD, or 2.0% SL/0.1% SD. Formulations were surface-inoculated, packaged, and stored as described above.

Preparation, inoculation, and sampling of meat products: lactate plus diacetate in bratwurst formulations. Two types of cooked bratwurst were evaluated: bratwurst that was cured and naturally smoked and bratwurst that was uncured and unsmoked. These products were produced by a commercial manufacturer. Smoked-bratwurst formulations included pork, water, beef, salt, corn syrup, sugar, monosodium glutamate, sodium erythorbate, sodium nitrite, and natural flavoring. Sodium nitrite was added to the batter at a rate of 156 ppm. Ingredients for unsmoked bratwurst included pork, water, salt, corn syrup, dextrose, natural flavoring, monosodium glutamate, and sodium phosphate. We evaluated smoked bratwurst (i) with no added lactate and diacetate and (ii) with 3.4% SL and 0.1% SD added. We evaluated unsmoked bratwurst (i) with 2.0% SL added and (ii) with 3.4% SL and 0.1% SD added. Products were surface-inoculated and packaged as described above for the wiener studies, but three links per package (approximately 227 g) were used. Bratwurst were stored at 3 or 7°C for up to 84 days.

Sampling and microbiological analysis. Three inoculated and two uninoculated packages per treatment were assayed at each sampling interval for changes in microbial populations and pH as described below. For trial 1 of the surface treatment study, wieners were assayed initially and after 1, 30 and 45 days of storage at 4.5°C. For the remaining wiener studies, products were assayed initially and after 7, 14, 30, 45, and 60 days of storage at 4.5°C. Bratwursts were assayed at initially; after 14, 28, and 42 days of storage at 3°C; and after 7, 14, and 28 days of storage at 7°C.

Bratwurst formulations that did not support pathogen growth at the end of the scheduled sampling intervals at either temperature were also assayed after 56 and 84 days.

Bacterial populations were determined from rinse material obtained after adding 100 ml of sterile Butterfield phosphate buffer to each package and massaging the contents externally by hand for about 3 min. *L. monocytogenes* was enumerated for triplicate inoculated samples by surface plating serial dilutions of rinse material on modified Oxford agar (37°C, 48 h). Select colonies were confirmed to be *L. monocytogenes* by Gram stain, tumbling motility, the CAMP test, hemolysis on Trypticase soy agar with sheep's blood, and biochemical analysis with MICRO-ID *Listeria* (Remel, Lenexa, Kans.).

Duplicate uninoculated samples were assayed for aerobic plate counts (plate count agar, 37°C, 48 h; Difco Laboratories, Detroit, Mich.) and psychrotrophic bacteria counts (Trypticase soy agar, 7°C, 7 days). After being rinsed for microbial assays, a 10-g portion of each sample was homogenized in 90 ml of hot water (ca. 90°C); the mixture was cooled, the fat layer was removed, and the pH was measured with a combination glass electrode (model 8104; Orion Research, Beverly, Mass.) and pH meter (Accumet Basic pH meter, Fisher Scientific, Chicago, Ill.).

Statistical analysis. One-way and two-way analyses of variance were performed on microbiological data with the Minitab v. 12.23 statistical software package (Minitab Inc., State College, Pa.) to determine statistically significant differences ($P < 0.05$) of mean values between treatments at each sampling interval and temperature, where applicable. The analysis included triplicate samples for each treatment at each sampling interval.

RESULTS

Surface treatment of wieners. Surface treatment of wieners via immersion in 6% SL for 2 min did not significantly ($P > 0.05$) delay listerial growth compared with the growth on the untreated control (Table 2). Populations of *L. monocytogenes* on wieners treated with 6% SL increased by 1.1 log₁₀ CFU per package at 30 days and by an additional 0.6 log₁₀ CFU per package at 45 days. In contrast, no growth was observed on day 30 on wieners immersed

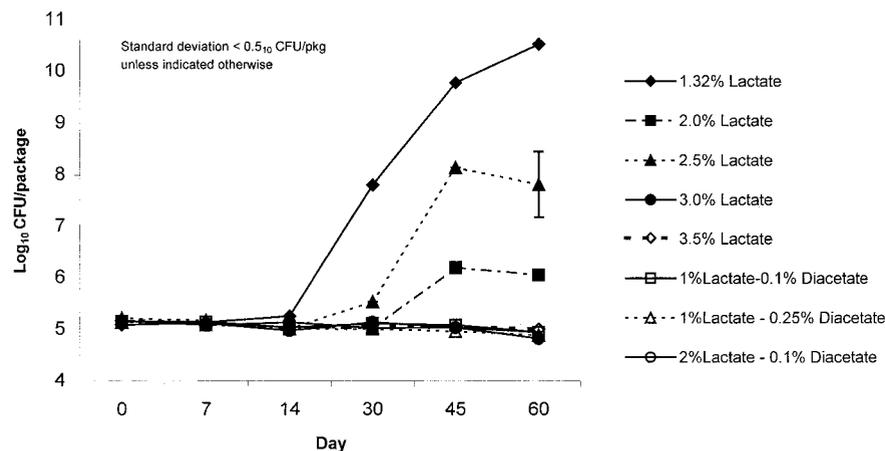


FIGURE 1. Inhibition of *L. monocytogenes* by SL and SD on cured, smoked wieners stored at 4.5°C. Population numbers reported are averages for triplicate packages.

in 3% SD, but the pathogen had increased by 0.6 log₁₀ CFU per package by day 45. Populations of *L. monocytogenes* on day 45 were 2.08 log₁₀ CFU per package smaller on wieners immersed in solutions containing 6% SL and 3% SD than on the control wieners ($P < 0.05$). In subsequent trials, we evaluated two levels of lactate-diacetate and a reduced exposure time that was more representative of commercial manufacturing conditions.

When the exposure time was reduced from 2 min to 5 s, neither of the lactate-diacetate surface treatments (6% SL and 3% SD, 3% SL and 1.5% SD) inhibited the growth of *L. monocytogenes* compared with that on untreated control wieners ($P > 0.05$) during 60 days of storage at 4.5°C (Table 2). After 14 days, pathogen levels remained unchanged from the initial inocula of 4.7 ± 0.1 log₁₀ CFU per package for all treatments. Sporadic growth (increases of 0.5 to 2 log₁₀ CFU per package) was detected on wieners sampled after 30 and 45 days of storage. After 60 days of storage, populations of *L. monocytogenes* increased to approximately 5.4 ± 0.7 , 5.9 ± 0.4 , and 5.2 ± 0.4 log₁₀ CFU per package for untreated control wieners, wieners treated with 6% SL and 3% SD, and wieners treated with 3% SL and 1.5% SD, respectively (Table 2).

The pH values of the wieners remained constant at 6.3 ± 0.03 throughout the testing interval regardless of surface treatment. Both aerobic and psychrotrophic bacterial populations for uninoculated control wieners averaged 3.86 ± 0.12 log₁₀ CFU per package initially (data not shown). Uninoculated wieners dipped in lactate-diacetate solutions exhibited slightly lower aerobic and psychrotrophic plate counts initially than did untreated wieners. Aerobic bacterial populations initially were 3.26 ± 0.68 log₁₀ CFU per package for treatment with 6% SL and 3% SD and 3.36 ± 0.25 log₁₀ CFU per package for treatment with 3% SL and 1.5% SD, whereas psychrotrophic bacterial populations were 2.89 ± 0.41 log₁₀ CFU per package for treatment with 6% SL and 3% SD and 3.53 ± 0.18 log₁₀ CFU per package for treatment with 3% SL and 1.5% SD. Aerobic and psychrotrophic bacterial populations increased to averages of 5.0 log₁₀ and 8.5 log₁₀ CFU per package for untreated control wieners after 30 and 45 days of storage, respectively. No additional increase was observed after 60 days. In contrast, after 60 days of storage, populations of aerobic and

psychrotrophic bacteria gradually increased to 7.4 log₁₀ CFU per package for treatment with 6% SL and 3% SD, compared with an increase to 4.9 log₁₀ CFU per package for treatment with 3% SL and 1.5% SD (data not shown).

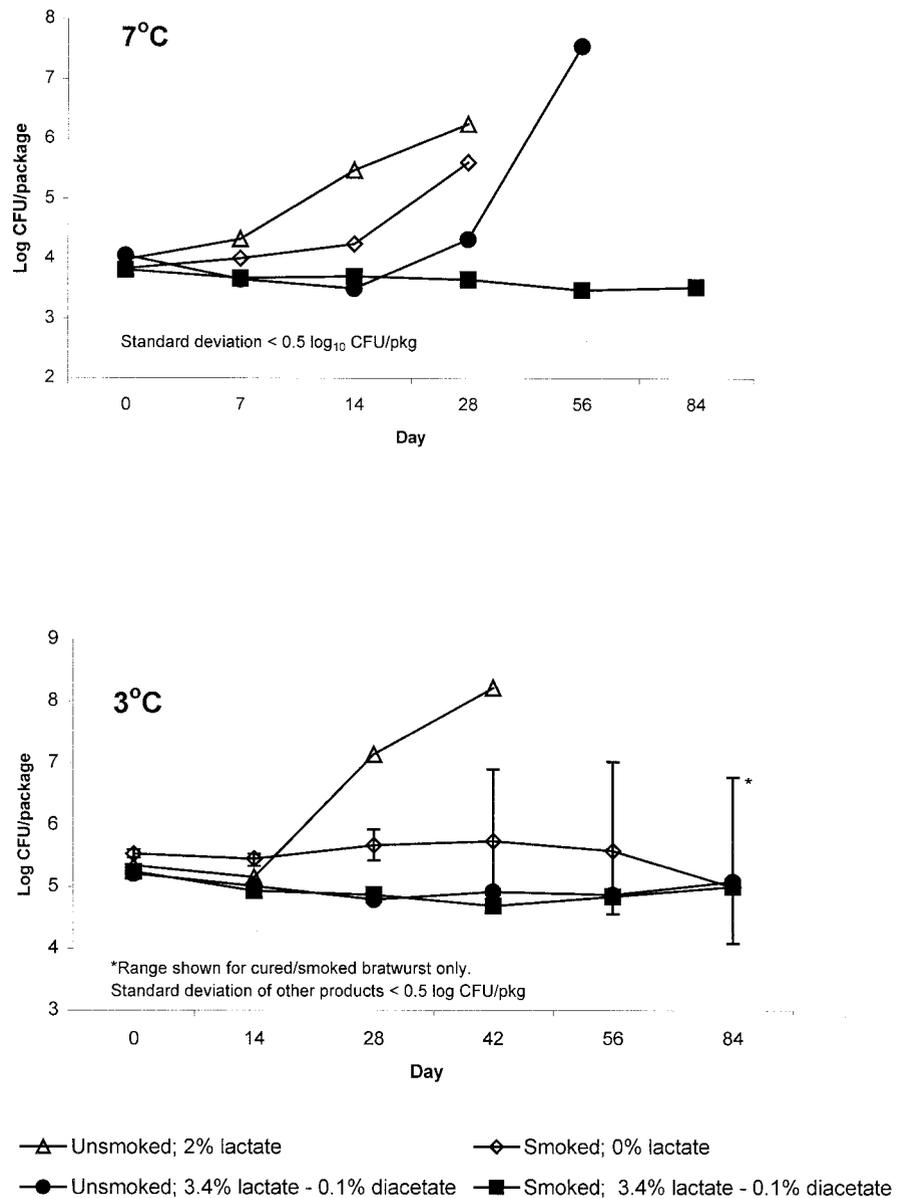
Lactate and diacetate in wiener formulations. Wieners formulated with 3.0 or 3.5% SL or combinations of $\geq 1.0\%$ lactate and $\geq 0.1\%$ diacetate inhibited the growth of *L. monocytogenes* for 60 days at 4.5°C (Fig. 1). However, the treatments were not listericidal; populations of *L. monocytogenes* did not decrease from the initial inoculum of 5.2 log₁₀ CFU per package with any formulation.

Wieners formulated with 1.32% SL supported the growth of *L. monocytogenes* when stored at 4.5°C for ≥ 30 days (Fig. 1). Populations of the pathogen increased by >2.5 log₁₀ CFU per package by day 30 and by an additional 2 log₁₀ CFU per package by day 45 and had grown to 10.5 log₁₀ CFU per package by day 60. Formulations containing 2.0 and 2.5% SL exhibited delayed growth until day 45. Populations of *L. monocytogenes* increased by 1 log₁₀ CFU per package for wieners formulated with 2.0% SL and by 2 to 3 log₁₀ CFU per package for wieners formulated with 2.5% SL by day 45. No additional increase in populations of *L. monocytogenes* was observed for either treatment on day 60.

The initial pH for the treatments ranged from 5.9 to 6.4 (Table 1). Formulations containing SD had slightly lower pH values than those that did not contain diacetate. The pH value of wieners formulated with 1.0% lactate plus 0.25% diacetate was 0.5 pH units lower than that of wieners formulated with 1.32% lactate alone and 0.2 units lower than that of wieners formulated with 1.0% lactate plus 0.1% diacetate. The pH range for all formulations was within the growth range for *L. monocytogenes* (19).

Standard plate counts for all uninoculated samples remained <3 log₁₀ CFU per package throughout the testing period except for sporadic packages that exhibited bacterial growth (data not shown). Aerobic plate counts were 6.1, 7.4, and 6.6 log₁₀ CFU per package on days 30, 45, and 60, respectively, for one of two uninoculated packages of wieners formulated with 2% lactate plus 0.1% diacetate. One uninoculated package of product formulated with 1% lactate and 0.25% diacetate contained 5.1 log₁₀ CFU per

FIGURE 2. Inhibition of *L. monocytogenes* by SL and SD on bratwurst stored at 7 and 3°C. Population numbers reported are averages for triplicate packages. Ranges for the pathogen are shown only when the standard deviations are $>0.5 \log_{10}$ CFU per package.



package on day 45. Standard plate counts did not correlate with the growth or inhibition of *L. monocytogenes* in any of the treatments.

Lactate and diacetate in bratwurst formulations.

Populations of *L. monocytogenes* for all treatments stored for 7 days at 7°C were similar to the initial inoculum of $3.9 \pm 0.1 \log_{10}$ CFU per package (Fig. 2). Unsmoked, uncured bratwurst formulated with 2% lactate supported an *L. monocytogenes* increase of $1.5 \log_{10}$ CFU per package by day 14 and an additional increase of $0.8 \log_{10}$ CFU per package by day 28. The presence of 3.4% lactate and 0.1% diacetate in unsmoked bratwurst inhibited listerial growth for 28 days, but numbers of the pathogen had increased to $7.6 \log_{10}$ CFU per package by day 56. Populations of *L. monocytogenes* increased by $0.4 \log_{10}$ CFU per package on smoked bratwurst without additives by day 14 and had increased to $5.6 \log_{10}$ CFU per package by day 28. In contrast, the pathogen did not grow on smoked bratwurst formulated with 3.4% lactate and 0.1% diacetate throughout 84 days of storage at 7°C.

No listerial growth was observed for any treatment after storage at 3°C for 14 days (Fig. 2). By day 28, populations of *L. monocytogenes* had increased on uncured, unsmoked sausage with 2% lactate from $5.3 \log_{10}$ to $7.1 \log_{10}$ CFU per package. Cured, smoked bratwurst without additives demonstrated the greatest variation, with one of three samples for this treatment supporting a listerial population increase of 1.2 to $1.7 \log_{10}$ CFU per package after 42, 56, and 84 days of storage. No listerial growth was observed for either product type formulated with 3.4% lactate plus 0.1% diacetate.

Except for smoked bratwurst without additives, psychrotrophic and standard plate counts did not increase for the treatments that inhibited listerial growth for 12 weeks (data not shown). Psychrotrophic and aerobic plate counts did not increase for smoked bratwurst formulated with lactate and diacetate stored at 7 or 3°C or for unsmoked bratwurst with lactate-diacetate stored at 7°C. Psychrotrophic and aerobic plate counts increased to $6.5 \log_{10}$ CFU per package for duplicate packages of uninoculated smoked

bratwurst stored at 3°C. The pH of these packages decreased from 6.2 to 5.1. Coincidentally, two of the three inoculated packages for this treatment inhibited pathogen growth. There was no obvious correlation among growth of spoilage organisms, decrease in pH, and listerial growth for any other formulation at either temperature.

DISCUSSION

Several combinations of SL and SD were evaluated as surface treatments for wieners and as part of the formulations for two types of cooked sausage products to determine their ability to inactivate or control the growth of *L. monocytogenes*. Our research revealed that formulation of the product with moderate levels of SL or certain combinations of lactate and diacetate prevented the growth of *L. monocytogenes* on the surface of smoked wieners and cooked bratwurst, but these formulations were not listericidal under the conditions of this study.

Smoked wieners formulated with 3 or 3.5% SL inhibited listerial growth throughout 60 days of storage at 4.5°C. Formulating the product with 2 or 2.5% lactate delayed listerial growth on smoked wieners stored at 4.5°C for 28 days, but 2% lactate was insufficient to inhibit pathogen growth on unsmoked bratwurst after 14 days' storage at 7°C. Schlyter et al. (28) similarly reported that 2.5% lactate increased the lag phase but did not prevent listerial growth in uncured, unsmoked turkey slurries stored at 4.4°C, and Shelef and Potluri (31) observed listeristatic activity of 3% lactate in cooked liver sausage stored at 5°C. Stillmunkes et al. (32) reported that there was no growth of *L. monocytogenes* on cooked beef roasts treated with 3.5% lactate and stored at 2 to 4°C for 5 weeks, but the pathogen increased by 0.9 log₁₀ and 2.7 log₁₀ CFU/cm² for 2.5 and 1.5% lactate treatments, respectively. These results are in contrast to those of a study by Bacus and Bontenbal (3) claiming that 2.0% lactate controlled the growth of *L. monocytogenes* in frankfurters for 6 weeks of storage at 4.4°C; 4% lactate was required to demonstrate comparable antilisterial activity in uncured cooked chicken breast meat.

Levels of individual antimicrobial agents can be reduced when diacetate and lactate are used together. In this study, combinations of ≥1.0% SL and ≥0.1% SD inhibited surface growth of *L. monocytogenes* on smoked wieners stored for 60 days at 4.5°C. These observations confirm previous work evaluating antimicrobial agents in refrigerated unsmoked turkey slurries (28). The presence of 2.5% lactate or 0.1% diacetate alone increased the lag phase but did not prevent listerial growth. However, *L. monocytogenes* was inhibited at 4.5°C for 42 days in refrigerated turkey slurries formulated with 2.5% SL and ≥0.1% SD.

The antimicrobial activity of lactate plus diacetate is enhanced in cured, smoked products compared with such activity in uncured, unsmoked meats. Combinations of 3.4% SL plus 0.1% SD delayed growth for 84 days for smoked, cured bratwurst stored at 7°C, compared with a 28-day delay for uncured, unsmoked bratwurst ($P < 0.05$). The enhanced activity is likely due to interactions with components provided by the smoking process or with the residual nitrite. Although smoke by itself is not considered

effective in controlling *L. monocytogenes* in seafood (15), other studies have suggested that compounds associated with smoke, such as phenols, formaldehyde, and acetic acid, have antilisterial properties (11, 25, 37). Phenol concentrations as low as 12.5 ppm inhibited the growth of *L. monocytogenes* in media supplemented with 2% NaCl and stored at 4°C, but a concentration of 20 ppm is required to prevent listerial growth at 8°C (33). Considerably higher phenol levels, ranging from 54 to 136 ppm, were reported to exhibit antilisterial activity in wiener exudates (37). The higher phenol concentrations were associated with a reduction of 4 log₁₀ CFU/ml in exudates stored at 4°C for 20 days, whereas the lower concentrations were listeristatic (37).

The cured products used in this study were formulated with 156 ppm nitrite, but the residual levels were <2 to 31 ppm after cooking and chilling. Significant reductions in nitrite levels are common after the heating of cured meat products. The low residual nitrite levels found in products used for this study are similar to the range of 1 to 10 ppm reported in a recent survey of retail wieners (7). Concentrations of nitrite that are typically found in finished cured meat products may be insufficient to significantly inhibit *L. monocytogenes* under the given pH, salt, and temperature conditions (5). No interaction was observed between 30 ppm nitrite and diacetate at levels as high as 0.5% in turkey slurries (28). In contrast, antilisterial activity was observed in smoked salmon formulated with 2% lactate, 3% NaCl in the water phase, and 125 ppm nitrite, whereas no inhibition was observed in smoked salmon formulated without nitrite (26).

A lower temperature enhanced the antilisterial activity of lactate and diacetate on the unsmoked bratwurst evaluated in this study ($P < 0.05$). No growth was observed at 3°C for 84 days on unsmoked bratwurst formulated with 3.4% lactate and 0.1% diacetate, but the same formulation allowed listerial growth after 28 days' storage at 7°C. These findings support previous reports that the minimum inhibitory concentrations of SL and SD decrease in relation to temperature reduction for several gram-positive bacteria (9, 24, 30, 31).

Competitive microflora may also contribute to the inhibition of *L. monocytogenes* in RTE meat products. Canadian researchers have reported that the growth of lactic acid bacteria was a significant factor in determining the inhibition of the pathogen in retail wieners (22). A certain minimal level of fermentable carbohydrate in the product may be necessary for the lactic acid bacteria to reduce the product pH significantly. In previous studies at the Food Research Institute, significant differences were observed in listerial growth between wieners produced by two different meat processors (17). One wiener brand containing 1.5% carbohydrates supported an *L. monocytogenes* increase of 5 log₁₀ CFU/g before spoilage, compared with an increase of 0.6 log₁₀ CFU/g for the second brand, which contained 2.8% carbohydrates. The pH decreased by 0.6 pH units and lactic acid bacteria populations increased by 4.8 log₁₀ CFU/g in the product with the higher carbohydrate level. In contrast, the pH remained unchanged and a lactic acid bacteria

increase of 2.8 log₁₀ CFU/g was observed for the lower-carbohydrate brand that supported listerial growth. In the surface treatment and bratwurst trials of this study, the growth of spoilage organisms did not appear to correlate with any significant pH decrease. SL has also been reported to inhibit spoilage bacteria (10, 18). Manufacturers must optimize the critical balance between increasing the shelf life and permitting the growth of spoilage lactic acid bacteria that compete with *L. monocytogenes* for nutrients.

CONCLUSIONS

Surface treatment of cooked meat products with antimicrobial solutions was evaluated in this study because this method delivers the antimicrobial agents at the point of contamination: after chilling and before packaging. Furthermore, surface application was deemed to have less impact on the sensory and functional properties of the product than inclusion of antimicrobial agents in the product formulation. However, our data suggest that dipping wieners into lactate-plus-diacetate solutions is not a reliable means to control listerial growth.

This study revealed that the inclusion of low levels of lactate and diacetate in the formulations of RTE meats can control the growth of *L. monocytogenes*. These antimicrobial agents are more effective in smoked products formulated with sodium nitrite or in products stored at strict refrigeration temperatures. Manufacturers should verify the efficacy of lactate and diacetate in uncured products to comply with the regulations of the U.S. Department of Agriculture's Food Safety and Inspection Service (13). Lactate and diacetate demonstrate listeristatic rather than listericidal activity and will not be effective against gross contamination of a product with *L. monocytogenes*. However, inclusion of these secondary barriers can enhance the safety of RTE meats when used in conjunction with diligent hazard analysis critical control point and environmental *Listeria* control programs.

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