Enhanced Inhibition of *Listeria monocytogenes* and *Salmonella Enteritidis* in Meat by Combinations of Sodium Lactate and Diacetate

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ABSTRACT

The antimicrobial activities of sodium lactate (SL) and sodium acetate (SA) are well documented, but there is limited information on the effect of their combination or of the combination of SL and sodium diacetate (SDA) on survival and growth of *Listeria monocytogenes* and salmonellae in meat. Effects of SL (1.8 and 2.5%), SDA (0.1 and 0.2%), or SA (0.2%) and their combinations on the behavior of *L. monocytogenes* and *Salmonella enterica* serovar Enteritidis were investigated in sterile comminuted beef (pH 6.3, 79% moisture) during storage at 5 and 10°C. Although *L. monocytogenes* grew faster than *Salmonella Enteritidis* in control samples at 10°C, numbers of both pathogens increased from 3.5 to approximately 8.0 log CFU/g after 20 days. SL (1.8%) decreased the growth rate of both *L. monocytogenes* and *Salmonella Enteritidis*. SDA (0.2%) was more effective than SL in decreasing the growth rate of *L. monocytogenes*, and it caused a more than 1 log CFU/g decline in initial numbers of *Salmonella Enteritidis* during storage for 25 days at 10°C. Synergy was observed by combinations of SL and SDA. Combinations of 2.5% SL and 0.2% SDA were bacteriostatic to *L. monocytogenes* and bactericidal to *Salmonella Enteritidis* after 20 days at 10°C. At 5°C, a listeriostatic effect was produced by 1.8% SL + 0.1% SDA, whereas numbers of *Salmonella Enteritidis* were less than 10 cells/g after refrigeration for 30 days. Although SA was consistently and significantly less inhibitory than SDA, its mixtures with SL also demonstrated synergistic activity against both pathogens. Combinations of 2.5% SL and 0.2% SDA can be expected to greatly enhance the safety of refrigerated and temperature-abused ready-to-eat meats.

Foodborne listeriosis, caused by *Listeria monocytogenes*, continues to be of major concern to the food industry and the general public because of its high mortality rate of more than 25% and its economic impact (7, 11, 12, 24, 26). Outbreaks have been associated with ready-to-eat foods such as coleslaw, milk, soft cheese, and meats (7, 11, 12, 17). Salmonellosis is another commonly reported foodborne infection, with *Salmonella enterica* serotype Enteritidis being the most frequently identified in foodborne infections in the United States (13, 23). Foods implicated in salmonellosis include eggs, poultry, meat, and raw produce (4, 10, 13, 23).

Ensuring the microbial safety and shelf life of foods depends on minimizing the initial level of microbial contamination, preventing or controlling the level of microbial growth, or destroying microbial populations. However, despite food safety regulations and Hazard Analysis Critical Control Points practices, the incidence of foodborne diseases, and particularly of listeriosis, has been increasing in the industrialized world in the last decade (6, 24).

Organic acids such as lactic and acetic acids are known for their antimicrobial effects in foods. The mechanism of inhibition has been attributed to entry of the undissociated form of the weak lipophilic acids into the microbial cells, dissociation within the cells, and acidification of the cell interior (8, 16). The salts of these two acids are generally recognized as safe and are approved as direct ingredients for use in foods. The sodium salt of lactic acid is used as a humectant and flavor enhancer in meat and poultry products and contributes to increased cooking yields and water-holding capacity (19). Levels of 2% (3.3% of the available 60% solution) have been recommended in meat and poultry products (19), although higher concentrations are now deemed more effective (25). Sodium lactate (SL) (2 to 3%) has been shown to control growth of *L. monocytogenes* (2, 21) and *Listeria innocua* (9) in meat, with minimal effect on pH and sensory characteristics of the products. Sodium diacetate (SDA) is a flavoring agent and an antimicrobial, and levels of 0.1 to 0.2% are recommended for use in meat products (3). At concentrations of 0.1 to 0.3%, SDA has been shown to control growth of *L. monocytogenes* in meat (18). Shelef and Addala (20) reported that SDA added to brain heart infusion (BHI) broth delayed growth of *L. monocytogenes*, and the effect was enhanced at a lower storage temperature. They further confirmed that concentration of 0.1 or 0.3% SDA suppressed growth of *Listeria* in fresh meat (20).

Synergistic effects of mixtures of lactic and acetic acids were reported in studies with foodborne pathogens such as *Salmonella Enteritidis* and *Escherichia coli* (1, 15). However, there is limited information on synergistic effects of salts of these acids on foodborne pathogens at or near pH
6. The purpose of the present study was to investigate the effect of SL, SDA, or sodium acetate (SA) and their combinations on the fate of L. monocytogenes and Salmonella Enteritidis in a sterile comminuted beef model system during storage at 5 and 10°C. The behavior of each of these pathogens in the meat, alone, or in combination was further compared.

MATERIALS AND METHODS

Microorganisms. L. monocytogenes Scott A serotype 4b, originally obtained from M. P. Doyle, University of Georgia, and Salmonella enterica serotype Enteritidis ATCC 13076 were used in the study. The cultures were maintained on BHI agar slants and grown in 5 ml of BHI broth at 35°C for 18 to 24 h before use. Serial dilutions of the fresh cultures were carried out in 0.1% peptone water (PW).

Meat and chemicals. A salt-free sterile comminuted beef emulsion, 79% moisture, 5% fat (Gerber, Fremont, Mich.), was used in the study. SL was obtained from PURAC America Inc. (Lincolnshire, Ill.); SA and SDA were from Niacet (Niagara Falls, N.Y.). All salts were food grade.

Preparation and inoculation of samples. Salt levels (% by weight) added to the beef were as follows: SL, 1.8 and 2.5%; SDA, 0.1 and 0.2%; or SA, 0.2%; and their combinations. The salts, in concentrated aqueous solutions, were thoroughly mixed into the meat (250-g batches). Equal amounts of sterile water (1.5 ml per 100 g) were added to the untreated meat that served as control. Samples (11 g) were dispensed into plastic cups (30-ml volume), tightly covered with aluminum foil, and sterilized for 15 min at 121°C. L. monocytogenes, Salmonella Enteritidis, or their combination (approximately 4 log CFU in 0.1 ml of PW) was added to each of the cooled samples and the content thoroughly mixed. Samples were stored at 5 or 10°C.

Enumeration of microorganisms and pH measurement. Cells were enumerated in the meat samples immediately after inoculation and at 5-day intervals until levels of approximately 8 log CFU/g were reached or for 25 days at 10°C and 30 days at 5°C. Samples (11 g), in duplicate, were combined with sterile PW (99 ml) in stomacher bags, and the contents blended for 2 min. Appropriate dilutions in PW were plated in duplicate on prepoured selective agar plates (PALCAM and XLT4 for L. monocytogenes and Salmonella Enteritidis, respectively). Colonies were counted after incubation of the plates at 35°C for 24 to 48 h. Meat pH was measured initially and at each sampling time by directly inserting the pH electrode (model 720A, Orion Research Inc., Boston, Mass.) into the meat homogenates (1:10 dilution in PW). This procedure provided readings comparable with those obtained by direct measurements of the meat pH. All microbiological media were from Difco Laboratories, Detroit, Mich.

Preenrichment of samples stored at 5°C. For the detection of surviving Salmonella Enteritidis, meat samples were preenriched in Salmonella selective broth as described by Peng and Shelef (14). The medium consists of Proteose Peptone no. 3, yeast extract, sodium chloride, ferric ammonium citrate, sodium thiosulfate, d-psicose, and xylose, lysine and ornithine, novobiocin, and Tergitol. Presence of salmonellae in the meat samples is identified by black coloration of the medium during incubation in the BioSys instrument at 42°C (14).

Data analysis. Each trial was repeated twice, and duplicate samples were tested at each sampling time. All data were analyzed by SPSS computer program, version 10.0 (22). Statistical methods included independent sample t test and one-way analysis of variance. Significance was based on a probability level of 0.05 (P < 0.05).

RESULTS

The initial L. monocytogenes and Salmonella Enteritidis populations in the meat samples were approximately 3.5 log CFU/g. These populations were selected so that increases or declines in counts over time could be measured. Although L. monocytogenes grew at a faster rate than Salmonella Enteritidis at 10°C, concentrations of approximately 8 log CFU/g of both pathogens were reached by day 20 in the untreated samples. The growth pattern of each pathogen was similar whether inoculated into the meat alone or in combination with the other L. monocytogenes in the untreated meat reached 8 log CFU/g after 30 days at 5°C, whereas Salmonella Enteritidis declined to undetectable numbers on XLT4 agar plates.

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The meat pH (6.3) was not affected by the addition of SL and SA and was slightly reduced to 5.9 after addition of SDA (P > 0.05). A small pH increase of 0.2 to 0.3 pH units was observed in both treated and untreated samples during storage for 20 days at 10°C, and no change was observed in the refrigerated samples. Hence, the pH range of the meat throughout the study remained well above the minimum required for growth of both pathogens.

SL (1.8 and 2.5%), SDA (0.1 and 0.2%), and SA (0.2%) caused a decrease in growth rate of L. monocytogenes during storage at 10°C, but cell numbers were not significantly different from those in control samples after 20 days. The behavior of L. monocytogenes in meat treated with 2.5% SL, 0.2% SA, or 0.2% SDA and in untreated meat is illustrated in Figure 1A. Combinations of SL (2.5%) and SA or SDA (0.2%), however, were more inhibitory (P < 0.05), and the combination of SL (2.5%) and SDA (0.2%) was listeriostatic (Fig. 1B).

Salmonella Enteritidis was more sensitive than L. monocytogenes to each of the salts. Concentrations of 0.2% SDA or SA inhibited growth of the organism at 10°C, but cell numbers were not significantly different from those in control samples after 20 days. The behavior of L. monocytogenes in meat treated with 2.5% SL, 0.2% SA, or 0.2% SDA and in untreated meat is illustrated in Figure 1A. Combinations of SL (2.5%) and SA or SDA (0.2%), however, were more inhibitory (P < 0.05), and the combination of SL (2.5%) and SDA (0.2%) was listeriostatic (Fig. 1B).

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The behavior of L. monocytogenes in the meat emulsion during storage at 5°C for 30 days is illustrated in Figure 3. Decreased growth rate showed in samples treated with SL (1.8 and 2.5%) and SDA (0.1%), whereas treatments with the combinations of these salt concentrations were listeriostatic. No Salmonella Enteritidis colonies could be recovered on XLT4 agar plates from any of the beef samples after storage for 30 days. However, preenrichment for 24 h at 42°C resulted in black coloration of the broth for each of the treatments, indicating survival of very low numbers of salmonellae in each of the refrigerated treatments.
FIGURE 1. Behavior of L. monocytogenes in meat emulsion at 10°C. (A) Effect of single salts. ●, control; ▲, 2.5% SL; △, 0.2% SA; ○, 0.2% SDA. (B) Effect of salt combinations. ●, control; ■, 0.2% SA + 2.5% SL; ○, 0.1% SDA + 2.5% SL; △, 0.2% SDA + 2.5% SL. Each value is the mean of two tests run in duplicate.

FIGURE 2. Behavior of Salmonella Enteritidis in meat emulsion at 10°C. (A) Effect of single salts. ●, control; ▲, 2.5% SL; △, 0.2% SA; ○, 0.1% SDA; △, 0.2% SDA. (B) Effect of salt combinations. ●, control; ■, 0.2% SA + 2.5% SL; ○, 0.1% SDA + 2.5% SL; △, 0.2% SDA + 2.5% SL. Each value is the mean of two tests run in duplicate.

FIGURE 3. Behavior of L. monocytogenes in meat emulsion at 5°C. ●, control; □, 1.8% SL; ■, 2.5% SL; ○, 0.1% SDA; △, 1.8% SL + 0.1% SDA; ●, 2.5% SL + 0.1% SDA. Each value is the mean of two tests run in duplicate.

DISCUSSION

The addition of SL to meat emulsion caused a decrease in the growth rate of L. monocytogenes. Populations of 7 log CFU/g or higher were reached in meat treated with 2.5% of the salt after 15 days at 10°C and after 30 days at 5°C (Figs. 1A and 3). Previous work in our laboratory showed that a higher concentration (4% SL) suppressed L. monocytogenes growth in sterile comminuted chicken held at 5 and 20°C (21). Salmonella Enteritidis was more sensitive to 2.5% SL, with populations of 7.1 log CFU/g reached after 25 days at 10°C (Fig. 2A). Gram-negative bacteria have shown greater sensitivity to organic acids and their salts than gram-positive bacteria (20).

SDA is a dry salt composed of SA and acetic acid at a 1:1 molar concentration. This compound was consistently more effective than SA, the salt of acetic acid, in inhibiting L. monocytogenes (P < 0.05). It was also a more effective antilisterial agent than SL at the concentrations used in this study (P < 0.05). Concentrations of 21 mM (0.3%) and 28 mM (0.4%) SDA have been shown to control growth of aerobes in ground beef and ground beef slurry during storage at 5°C (20).
At 10°C, each of the tested salts proved ineffective in inhibiting growth of *L. monocytogenes* during storage. Although the higher concentration of SDA (0.2%) was most effective, *L. monocytogenes* populations were not significantly different from those in control and in the other salt treatments after storage for 20 days (Fig. 1A). When SL was combined with either SA or SDA, enhanced inhibition of *L. monocytogenes* was observed. Figure 4 illustrates the synergy for 2.5% SL and either 0.1 or 0.2% SDA. The bars represent the difference between log CFU/g in controls and in the respective treatments after 20 days at 10°C. Although the effects of 0.1 and 0.2% SDA alone were not significantly different (*P > 0.05*), their combination with 2.5% SL dramatically enhanced the antilisterial effect. The reduction of log CFU/g was 3.7 times (2.5% SL + 0.1% SDA) and 4.5 times (2.5% SL + 0.2% SDA) larger than the sum of reductions obtained with the single salts. Enhanced antilisterial effects were observed by the salts during storage at 5°C, and the combination of 1.8% SL and 0.1% SDA was listeriostatic during storage for 30 days. Higher concentrations (2.5% SL + 0.1 or 0.2% SDA) were only slightly more inhibitory. Used together, the effect of 1.8% SL and 0.1% SDA was 1.3 times higher than the sum of reductions obtained with the single salts (Fig. 5). Synergy, although less pronounced, was also observed by the combination of SL and SA. Schlyter et al. (18) reported concentrations of 0.1 or 0.3% SDA with 2.5% SL to be more effective in inhibiting growth of *L. monocytogenes* at 4 and 25°C compared with 0.1 or 0.3% SDA alone.

*Salmonella* Enteritidis was more sensitive than *L. monocytogenes* to all three salts tested during storage at 10°C, and similar to the effects on *L. monocytogenes*, inhibition was SDA (0.2%) > SA (0.2%) > SL (2.5%).

At a higher concentration of 0.2%, SA had a bacteriostatic effect, whereas SDA caused a slow decline in the initial cell numbers after 20 days of storage (Fig. 2A). Growth of *Salmonella* Enteritidis was inhibited by the combination of SL and SDA, and no colonies could be recovered on agar plates from meat treated with 2.5% SL and 0.2% SDA after 20 days at 10°C. However, preenrichment of these samples indicated survival of *Salmonella* Enteritidis cells. Survival of *Salmonella* Enteritidis was similarly detected in each of the treatments stored at 5°C.

SDA (0.1%) is used as a flavoring agent and antimicrobial in meat. Recently, the Food Safety Inspection Service, U.S. Department of Agriculture, approved a higher SDA concentration (0.25%) to inhibit growth of *L. monocytogenes* (25). The use of 4.8% SL as a flavoring agent and as a means of inhibiting certain pathogens in cooked meat and poultry products was also approved. However, the agency stated that no evidence was available for the efficacy of these high concentrations of the salts to inhibit pathogens. Our studies demonstrate that the combination of SL and SDA, at concentrations lower than the maximum approved for use and well within those considered acceptable organoleptically, was bactericidal to *Salmonella* Enteritidis at 10°C, bacteriostatic to *L. monocytogenes* at 10°C, and more inhibitory at refrigeration temperatures.

The inhibitory effects of the salts used in this study are not dependent on pH reduction, since the meat pH remained at approximately 6 throughout the storage periods. Therefore, other mechanisms, different from those proposed for their acids (8, 16), are responsible for the inhibitory effects of these salts. Although the mechanism of synergy of the salts is not yet understood, it was demonstrated here in an additive-free, low-fat, sterile beef emulsion. Equal or superior inhibition of listeriae and salmonellae can be expected in ready-to-eat (RTE) meats that contain sodium chloride, nitrates, and other antimicrobial additives. Enhanced effect of the inhibitory system is further predicted in most RTE meat products because of their higher fat content (compared with the 5% in the meat emulsion tested in this study) and the partition of the inhibitors in the water fraction of the foods. Moreover, since *Salmonella* Enteritidis is associated with poultry and eggs, the use of the antimicrobials combination can be extended also to RTE foods containing such products. Testing the effect of the

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**FIGURE 4.** Effect of SL (2.5%), SDA (0.1 and 0.2%), and their combination on the viability of *L. monocytogenes* in meat. The data represent the difference between populations in the untreated meat and in the respective treatments after 20 days at 10°C.

**FIGURE 5.** Effect of SL (1.8%), SDA (0.1%), and their combination on the viability of *L. monocytogenes* in meat. The data represent the difference between populations in the untreated meat and in the respective treatments after 30 days at 5°C.
inhibitory system on the two pathogens and the indigenous microorganisms in RTE products is under way.

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