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Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna

E. Mbandi, L.A. Shelef*

Department of Nutrition and Food Science, College of Science, Wayne State University, Detroit, MI 48202, USA

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Abstract

The antimicrobial activities of salts of organic acids such as lactate and acetate are well documented, but there is limited information on their effect when used in combination. We previously reported enhanced inhibition of *Listeria monocytogenes* and *Salmonella enterica* serovar Enteritidis in sterile comminuted beef at 5 and 10 °C by combinations of sodium lactate (SL) (2.5%) and sodium diacetate (SDA) (0.2%). The present study was undertaken to evaluate the inhibitory effect of these salts, alone and in combination, in ready-to-eat (RTE) meat. Single strains and six-strain mixtures of each of the pathogens (~ 3 log CFU/g) were tested in beef bologna during aerobic storage at 5 and 10 °C for up to 60 days. The growth rate of the six-strain mixture of *Listeria* was faster than that of the single strain (Scott A) in the lactate/diacetate-free product. While each of the salts delayed growth of the listeriae at 5 °C, the effect of their combination was listericidal for the single strain and listeriostatic for the six-strain mixture. Enhanced inhibition by the salt combination was also observed at 10 °C. *Salmonella* numbers declined to undetectable levels in the untreated meat product and in each of the treatments after 20–30 days. However, the decline was more rapid in meat with the combination of the salts during storage at both 5 and 10 °C. Each of the salts further delayed the growth of the background microflora during storage at 5 °C, with their combinations showing the most effect.

Keywords: *Listeria monocytogenes*; *Salmonella*; Antimicrobials; Lactate; Diacetate

1. Introduction

Meat products are among the leading foods associated with listeriosis and salmonellosis. *Listeria monocytogenes* is a gram-positive facultative anaerobe. It is ubiquitous in nature and common in foods of both plant and animal origin. Outbreaks of human listeriosis have resulted from the consumption of meat products contaminated with this pathogen (McLauchlin et al., 1991; Anon, 1999; Ryser and Marth, 1999). Due to the ubiquitous nature of this pathogen in the slaughterhouse and the meat packaging environments, it is not surprising that the incidence and behavior of this pathogen in meat products are receiving increasing attention. Salmonellae are gram-negative rods that are widely distributed in nature, with humans and animals being their primary reservoirs.

* Corresponding author. Tel.: +1-313-577-2998; fax: +1-313-577-8616.

E-mail address: lshelef@sun.science.wayne.edu (L.A. Shelef).

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Eggs, poultry, meat and meat products are the most common vehicles of salmonellosis to humans (D'Aoust, 1989; Mishu et al., 1994; Klontz et al., 1995), and *Salmonella enterica* serovar Enteritidis is one of the most frequently identified serovar in foodborne salmonellosis outbreaks in the United States (Frenzen et al., 1999).

Contamination of ready-to-eat (RTE) meats with *L. monocytogenes* and *Salmonella* spp. occurs mainly at postprocessing, and consumption of these products without further heating is common. Although RTE meats contain salts such as sodium chloride, nitrite and nitrate that have antimicrobial activities, they do not inhibit the growth of *L. monocytogenes* during storage at refrigerated temperatures. To simulate post-processing contamination, Glass and Doyle (1989) inoculated the surface of several commercially processed meat products with *L. monocytogenes* and vacuum-packaged the samples. They observed that after 6 weeks of refrigeration storage (4.4 °C), regardless of the inoculum number, *L. monocytogenes* populations increased by 10^3–10^4 CFU/g on organoleptically acceptable bologna, and a 10^3–10^5 CFU/g increase was observed in chicken and turkey products after 4 weeks of storage. This indicated that manufacturers cannot rely on the combination of vacuum packaging and refrigeration to control listeriae in RTE meats. Methods such as modified atmosphere packaging (MAP) (Avery et al., 1994) and bacteriocins (McMullen and Stiles, 1996) have been used to control *L. monocytogenes* in meat. The sodium salts of short-chain organic acids such as citric, acetic, lactic or their combinations have shown antimicrobial properties (Buchanan et al., 1993; Schlyter et al., 1993; Shelef and Addala, 1994; Shelef et al., 1997; Stekelenburg and Kant-Muermans, 2001). These generally recognized as safe (GRAS) ingredients in meat products could serve as additional hurdles to control or prevent proliferation of listeriae or other foodborne pathogens.

In a previous study, we reported enhanced inhibition of *L. monocytogenes* and *Salmonella* Enteritidis by combinations of sodium lactate and diacetate at 5 and 10 °C using a meat model system. The meat consisted of additive-free, sterile comminuted beef (79% moisture), and one strain of each pathogen was tested (Mbandi and Shelef, 2001). The combined concentration of 2.5% lactate and 0.2% diacetate was identified as an effective antimicrobial in the product. RTE meats contain a number of added ingredients that may affect the antimicrobial effects of the tested salts. Most also contain less moisture. Moreover, tests with one strain of a pathogen may not provide sufficient information on the behavior of the organism due to variability in sensitivity among strains. The present study was undertaken to evaluate the effect of the salts in commercial beef bologna containing approximately 57% moisture. Sodium lactate, diacetate and their combination were tested using single strains and six-strain mixtures of each of the pathogens. In addition, the effect of the salts on total aerobes in the product was evaluated.

2. Materials and methods

2.1. Microorganisms

The microorganisms used in this study were: *L. monocytogenes* Scott A (serotype 4b), V-7 (1), 10403S (1), EGD (1/2a), ATCC 19117 (4d) and *L. innocua* (ATCC 33090); *S. choleraesuis* subsp. *arizonae* (ATCC 29933), *S. enterica* serotype Enteritidis (ATCC 13076), *S.* Heidelberg (ATCC 1964), *S.* Newport (ATCC 6962), *S.* Typhimurium (ATCC 13311) and *S.* Dublin (ATCC 15480). Cultures of the organisms were maintained on brain heart infusion (BHI) agar slants and grown overnight in BHI broth at 35 °C before use. Serial dilutions of the fresh cultures were carried out in 0.1% peptone water (PW).

2.2. Meat and chemicals

Beef bologna (Eckrich, Downers Grove, IL) used in this study was obtained from local markets. Sodium lactate (SL) was from PURAC America (Lincolnshire, IL) and sodium diacetate (SDA) was from Niacet (Niagara Falls, NY).

2.3. Preparation and inoculation of bologna samples

Salt levels (percent by weight) added to the beef were: SL, 2.5%; SDA, 0.2%; and the combination of SL and SDA. The salts, in concentrated aqueous solutions, were thoroughly mixed with the meat in 250-g batches. Equal amounts of sterile water (1.5 ml/

100 g) were added to the untreated meat that served as control. Samples (11 g) were dispensed into plastic cups (30-ml volume) prior to inoculation with the test organisms. The effect of the salts on *Listeria* and *Salmonella* organisms was studied with a single or multiple-strain mixture of the pathogens. For the former, ~ 3 log CFU in 0.1 ml of PW of either *L. monocytogenes* Scott A or *S. enterica* serotype Enteritidis were added to each of the samples and the contents were thoroughly mixed. For the latter, a six-strain mixture of *Listeria* or *Salmonella* was used. Each strain was grown in 5 ml BHI broth for 18–24 h before use. Each culture (1 ml) was transferred into a 10-ml sterile test tube and mixed thoroughly. Serial dilutions were carried out with 0.1% PW, and 0.1 ml (~ 3 log CFU) was used to inoculate the meat samples as before.

2.4. Enumeration of microorganisms and pH measurement

Cell numbers in the meat samples were determined immediately after inoculation, and at 5–10- or 10–15-day intervals for up to 60 days at 10 or 5 °C, respectively. Meat samples (11 g) were combined with sterile PW (99 ml) in stomacher bags and the contents blended for 2 min (Stomacher 400; Seward Medical, London, UK). Appropriate dilutions in PW were plated in duplicate on prepoured selective agar plates (PALCAM and XLT4 for *Listeria* and *Salmonella*, respectively). Total aerobes in the samples were enumerated on prepoured plate count agar (PCA). Colonies were counted after incubation of the plates at 35 °C for 24–48 h. The meat pH was measured initially and at each sampling time by directly inserting the pH electrode (model 720A; Orion Research, Boston, MA) into the meat homogenates (1:10 dilution). All microbiological media were from Difco Laboratories (Detroit, MI).

2.5. Detection of low numbers of the pathogens

For the detection of low numbers of surviving cells, the meat samples were further incubated in Salmonella or Listeria Selective Broth for 24 h at 42 or 35 °C, respectively (Tan and Shelef, 1999; Peng and Shelef, 2000). The Salmonella Selective Broth contains carbohydrates and amino acids for selective detection of foodborne *Salmonella*, which include dulcitol and xylose as carbohydrate sources, and lysine and ornithine to identify decarboxylase activity (Tan and Shelef, 1999). The detection of listeriae is based on the hydrolysis of esculin to 6,7-dihydroxycoumarin (esculetin) and its reaction with ferric ions in the medium (Peng and Shelef, 2000). Presence of the pathogens in these media was identified by black discoloration, usually within 24 h. Detection by these procedures was confirmed using the official USDA procedures that were tested in parallel (Tan and Shelef, 1999; Peng and Shelef, 2000).

2.6. Data analysis

Each trial was repeated twice and duplicate samples were tested at each sampling time. An independent sample *t*-test was used to compare the treatments, and a one-way ANOVA was used to analyze the other variables (SPSS Version 10.0).

3. Results and discussion

3.1. Effect of the salts on pH

The initial pH of the bologna samples was 6.3. Addition of lactate did not affect the meat pH, but diacetate reduced it to 5.9. The combination of lactate and diacetate increased the meat pH to 6.1. Similar effects were reported earlier in studies with other meat products (Mbandi and Shelef, 2001; Stekelenburg and Kant-Muermans, 2001). There was a slow increase in pH in all samples during storage and the change was less than one pH unit by the end of the 60-day storage period.

3.2. Listeriae

The initial listeriae in the meat samples were ~ 3 log CFU/g and numbers in untreated samples exceeded 7 log CFU/g after 45 days at 5 °C. Although each of the salts delayed growth at 5 °C for about 30 days, the salt combination inhibited growth of the pathogen. A sharp decline in cell numbers was observed in tests with the single strain (Fig. 1A). Whereas no colonies could be recovered on PALCAM agar plates after 45 days, survival of very low

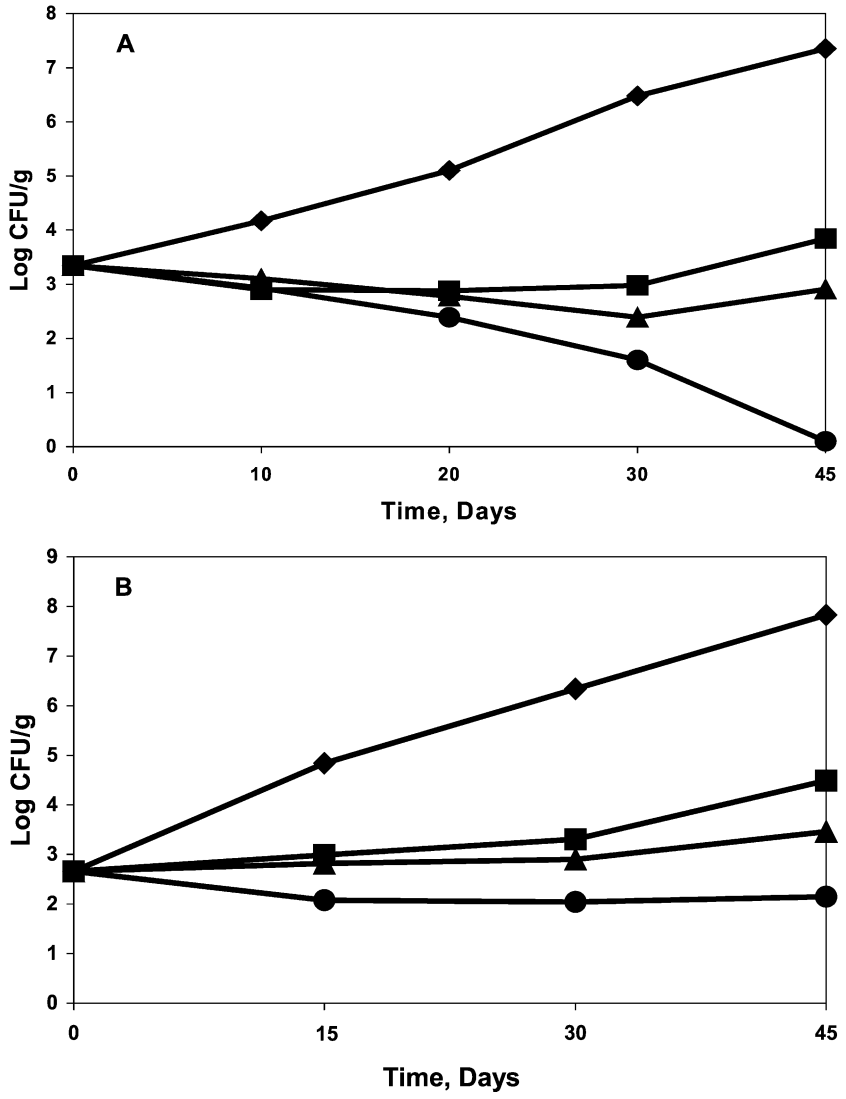

Fig. 1. Effect of sodium lactate (SL), diacetate (SDA) or their combination (SL + SDA) on listeriae in RTE meat at 5 °C. (A) Effect on *L. monocytogenes* Scott A. ♦: Control; ■: 2.5% sodium lactate; ▲: 0.2% sodium diacetate; ●: 2.5% sodium lactate + 0.2% sodium diacetate. (B) Effect on six strains of listeriae. ♦: Control; ■: 2.5% sodium lactate; ▲: 0.2% sodium diacetate; ●: 2.5% sodium lactate + 0.2% sodium diacetate.

numbers of listeriae was confirmed following incubation of samples for 24 h in the Listeria Selective Broth. A similar pattern of inhibition by each of the salts was observed in meat inoculated with the six-strain mixture. However, the salt combination was less inhibitory, resulting in a listeriostatic effect (Fig. 1B).

At 10 °C, numbers of the listeriae in untreated samples exceeded 8 log CFU/g after 45 days. As in studies at 5 °C, the combination of lactate and diacetate exhibited more pronounced growth inhibition than each of the salts alone, and the inhibitory effect on the single strain was superior to that on the six-strain mixture (Fig. 2A and B, respectively).

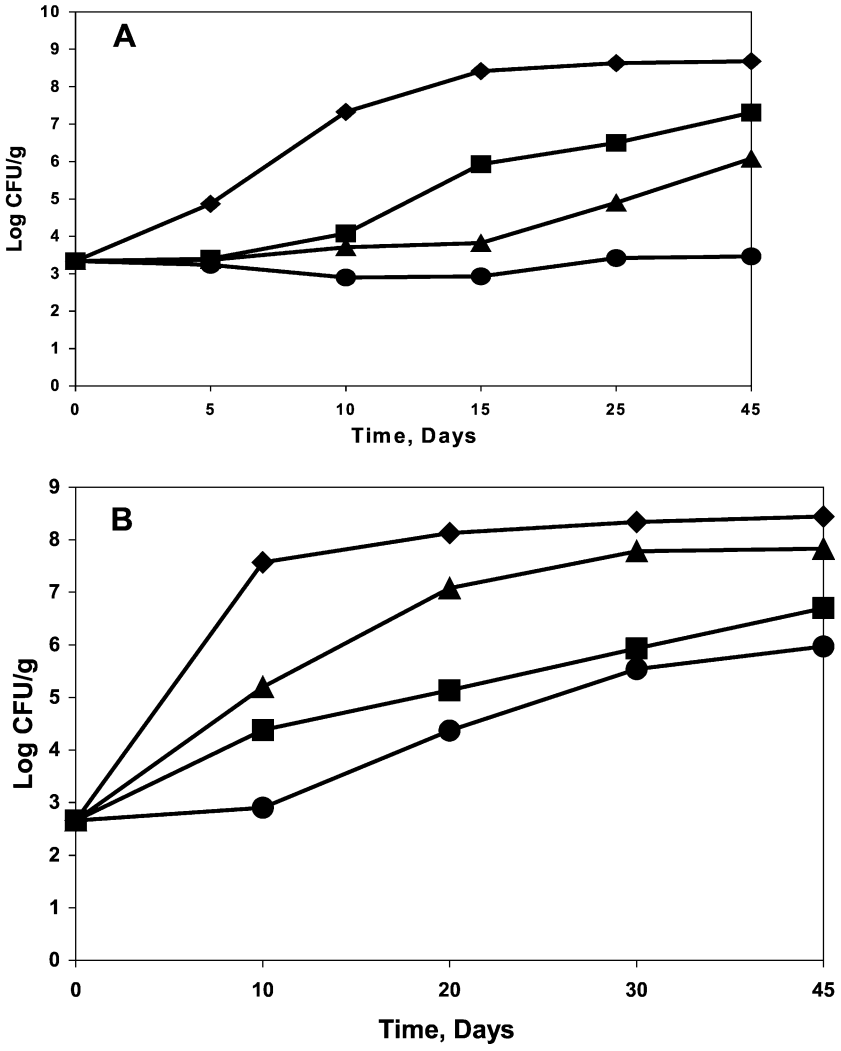

Fig. 2. Effect of sodium lactate (SL), diacetate (SDA) or their combination (SL + SDA) on listeriae in RTE meat at 10 °C. (A) Effect on *L. monocytogenes* Scott A. ♦: Control; ■: 2.5% sodium lactate; ▲: 0.2% sodium diacetate; ●: 2.5% sodium lactate + 0.2% sodium diacetate. (B) Effect on six strains of listeriae. ♦: Control; ■: 2.5% sodium lactate; ▲: 0.2% sodium diacetate; ●: 2.5% sodium lactate + 0.2% sodium diacetate.

3.3. Salmonellae

Salmonella numbers declined to undetectable levels during storage at both 5 and 10 °C in both treated and untreated samples, and the decline was most rapid in the presence of the combination of lactate and diacetate. Fig. 3A and B illustrates changes in cell numbers of *Salmonella* Enteritidis during storage at 5 and 10 °C, respectively. Similar results were observed for the six-strain mixture of salmonellae (data not shown). Testing the samples in the selective enrichment broth consistently indicated survival of very low numbers of the pathogen, even when no colonies could be recovered on agar plates.

3.4. Total aerobes

Mean initial total aerobes in the beef bologna samples (day 0) were 3.75 log CFU/g. Changes in their numbers in the presence of *L. monocytogenes*

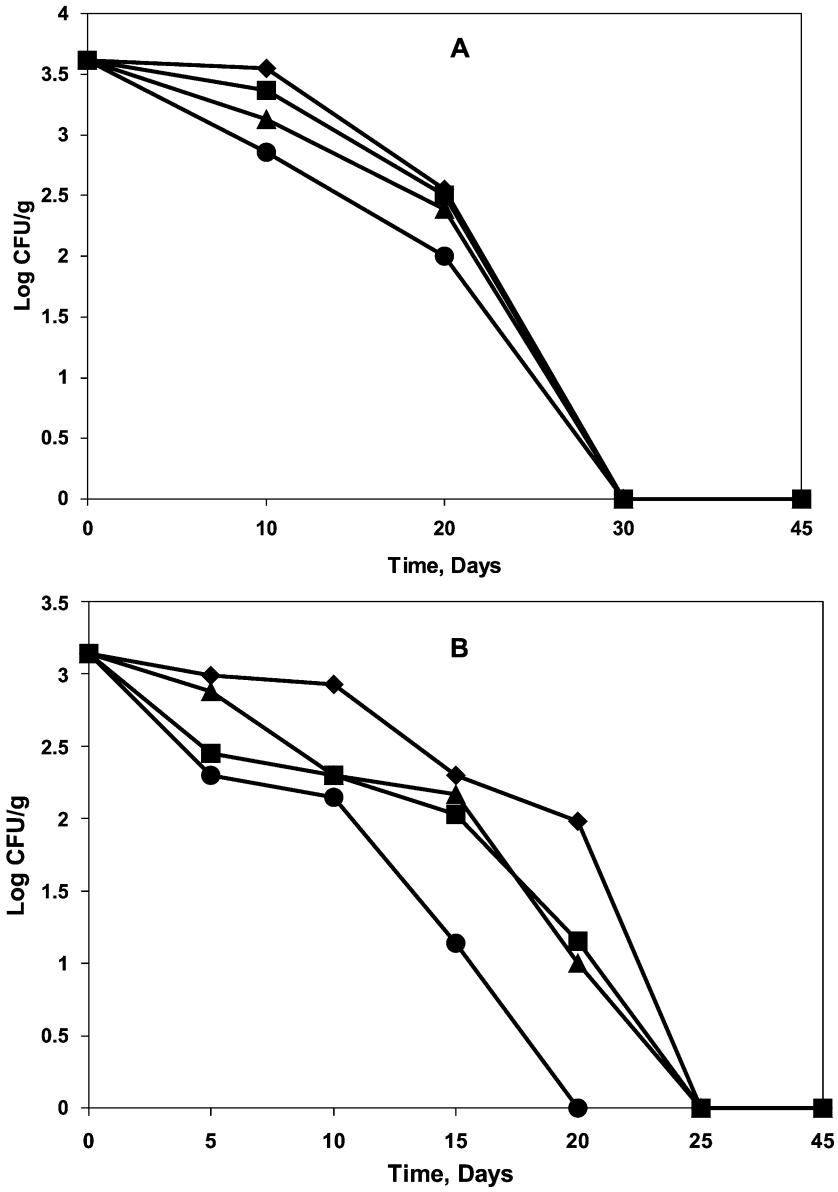

Fig. 3. Effect of sodium lactate (SL), diacetate (SDA) or their combination (SL + SDA) on salmonellae in RTE meat. (A) Effect on *Salmonella* Enteritidis at 5 °C. ♦: Control; ■: 2.5% sodium lactate; ▲: 0.2% sodium diacetate; ●: 2.5% sodium lactate + 0.2% sodium diacetate. (B) Effect on six strains of salmonellae at 10 °C. ♦: Control; ■: 2.5% sodium lactate; ▲: 0.2% sodium diacetate; ●: 2.5% sodium lactate + 0.2% sodium diacetate.

Scott A, *S.* Enteritidis and the six-strain mixture of each of the pathogens in samples treated with 2.5% SL, 0.2% SDA and their combination after storage for 25 days at 5 and 10 °C are summarized in Table 1. In most tests, total aerobes were at least 1 log higher than the pathogens. Hence, dilution of samples before plating on PCA eliminated the pathogens. In a few tests with *Listeria*, total aerobes were obtained by subtracting numbers of the pathogen enumerated on PALCAM from the total CFUs enumerated on the PCA plates. A

Table 1
Changes in populations of total aerobes in the presence of *L. monocytogenes* Scott A, *Salmonella* Enteritidis and the six-strain mixture of each of the pathogens in beef bologna treated with 2.5% sodium lactate (SL), 0.2% sodium diacetate (SDA) and their combination after storage for 25 days at 5 and 10 °C

Treatment	Log CFU/g (day 25 − day 0)							
	Strain Scott A		Listeriae mixture		*S.* Enteritidis		Salmonellae mixture	
	5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	5 °C	10 °C
Control	2.70[a]	4.97[a]	2.58[a]	4.01[a]	2.10[a]	3.79[a]	1.30[a]	3.34[a]
SL	1.32[b]	3.40[b]	1.00[b]	3.36[b]	1.77[a]	3.49[b]	0.63[b]	2.78[b]
SDA	2.03[c]	3.28[b]	1.63[c]	3.70[b]	1.17[b]	3.63[a]	0.65[b]	2.81[b]
SL + SDA	0.80[d]	3.23[b]	− 0.11[d]	3.41[b]	0.59[c]	3.23[b]	− 0.01[c]	2.49[b]

Values ± SD for duplicate samples of 2 trials. Different letters within a column denote significant difference ($P < 0.05$).

delay in growth of total aerobes was observed in the presence of sodium lactate or diacetate, and the inhibitory effect of these salts was enhanced when their combination was used. At 5 °C, the effect of the salt combination was significantly different from that of the other treatments ($P < 0.05$). On the average, < 0.5 log increase in total aerobes was observed in bologna treated with the salt combination during refrigeration for 25 days compared to increases of 1.2–1.4 log for the single salt treatments and over 2-log increase in controls. In most cases, the effect of the salt combination at 10 °C was not significantly different ($P > 0.05$) from that of lactate or diacetate treatment alone. Cell numbers in each of the salt-treated samples at this storage temperature were about 1 log lower than in the untreated samples ($P < 0.05$) after 25 days (Table 1).

4. Conclusions

Although lactate or diacetate alone exhibited antilisterial activity in the tested RTE meat, enhanced inhibition was observed at both storage temperatures when the salt combination was used. In particular, *Listeria* numbers declined or remained unchanged during 45 days of storage at 5 °C. Numbers of *Salmonella* organisms declined in the RTE meat at both 5 and 10 °C, whether treated or untreated with the salts. However, the decline was faster in the presence of the salt combination. Increases in total aerobes in the meat were also influenced by the tested salts, and the salt combination was significantly more effective than the single salts in controlling growth during refrigerated storage. These data confirm our previous findings of enhanced inhibitory activity of the combination of these salts in additive-free, sterile meat emulsion.

The tested salts were more inhibitory to the single strain of the pathogen, inferring differences in resistance of strains and the need for testing multistrain mixtures in the evaluation of antimicrobial agents.

Of the two pathogens tested in this study, *L. monocytogenes* can present a serious hazard to the consumer in both refrigerated and temperature-abused RTE meats. Our data show that proliferation of the pathogen can be prevented during storage at 5 °C by the combination of SL (2.5%) and SDA (0.2%). These levels are within those permitted for use as antimicrobials and flavoring agents in RTE meats in the US (USDA-FSIS, 2000). The combination further controls growth of the aerobic microorganisms in the product and hence prolongs its shelf life during aerobic storage conditions (i.e. after packages are opened). Although there was no increase in *Salmonella* numbers during refrigerated storage at 5 or 10 °C, the slow decline rate observed in this study, even in the presence of the salt combination, emphasizes the importance of avoiding contamination of RTE meats with this pathogen.

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