

Research Note

Survival and Growth of *Listeria monocytogenes* in Broth as a Function of Temperature, pH, and Potassium Lactate and Sodium Diacetate Concentrations

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ABSTRACT

The objective of this study was to determine the antimicrobial effect of a combination of potassium lactate and sodium diacetate (0, 1.8, 3, and 4.5%; PURASAL P Opti.Form 4, 60% solution) on the survival and growth of *Listeria monocytogenes* Scott A in pH-adjusted broth (5.5, 6.0, 6.5, and 7.0) stored at 4, 10, 17, 24, 30, and 37°C. Appropriate dilutions of broth were enumerated by spiral plating on tryptose agar and counted with an automated colony counter. Growth data were iteratively fit, using nonlinear regression analysis to a three-phase linear model, using GraphPad PRISM. At pH 5.5, the combination of lactate-diacetate fully inhibited ($P < 0.001$) the growth of *L. monocytogenes* at all four levels and six temperatures. At pH 6.0, addition of 1.8% lactate-diacetate reduced ($P < 0.001$) the specific growth rate of *L. monocytogenes* and increased lag time; however, 3 and 4.5% completely inhibited the growth at the six temperatures studied. Efficacy of the lactate-diacetate mixture was decreased as pH increased and incubation temperature increased. Thus, at pH 6.5, at least 3% was required to retard ($P < 0.001$) the growth of *L. monocytogenes* in broth. There was a limited effect of the lactate-diacetate level on the specific growth rate of the pathogen at pH 7.0. However, 1.8 and 3% significantly lengthened the lag time at 4 and 10°C. These results suggest that 1.8% of lactate-diacetate mixture can be used as a substantial hurdle to the growth of *L. monocytogenes* when refrigerated temperatures are maintained for products with pH less than 6.5.

Listeria monocytogenes is a relatively thermotolerant gram-positive, non-spore-forming bacterium that can cause listeriosis, which usually occurs in certain well-defined, high-risk groups including pregnant women, neonates, and immunocompromised adults. However, listeriosis may occasionally occur in persons with no predisposing underlying conditions. In nonpregnant adults, *L. monocytogenes* primarily causes septicemia, meningitis, and meningoencephalitis, with a mortality rate of 20 to 25% (29). This organism continues to be one of the most important foodborne psychrotrophic pathogens due to its ability to survive in diverse environmental conditions, such as low pH, pasteurization, and high NaCl concentrations (6). Due to the severity and case fatality rates of listeriosis in the highly susceptible human populations, the development of control measures for this pathogen is still of major interest to the food industry.

L. monocytogenes growth is highly dependent on product characteristics, including pH and temperature. The organism tends to grow well on meat products with pH values near or above 6.0, whereas it grows poorly or not at all on meat products near or below pH 5.0 (6). Many investigators

have shown that organic acids and their salts can inhibit the growth or accelerate the inactivation of the pathogen (7, 8, 11, 12, 21, 27, 28, 31, 32). The antimicrobial activity of the organic acids and/or their salts is dependent on many factors, such as temperature, pH, and concentration of the acid.

Sodium and potassium lactates and sodium diacetate salts have been recognized as safe additives for different meat products. They are already widely used in the food industry to extend the shelf life and increase the safety of meat and poultry products (1, 12, 15, 18). Lactates are clear, syrupy liquids derived from lactic acid, an acid naturally present in animal tissues. Sodium and potassium lactates can be used in meat products at levels from 2 to 4%, without adversely affecting sensory quality (30). These additives not only enhance meat and poultry flavor, but also provide one of the most effective antimicrobial hurdles for *L. monocytogenes* in meat products (7, 9–11, 23). The antimicrobial mechanism of lactates has at least two modes of action (5, 23). First, depression of the water activity of the product by the hygroscopic nature of sodium lactate; the salt chemically binds water molecules and, thus, retards development of spoilage and pathogenic microorganisms. Second, lactic acid in its undissociated form acts as a bac-

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teriostatic by interfering with the metabolism of bacteria and causing intercellular acidification, thereby increasing the lag phase. A rather new development in this field is the use of a combination of sodium or potassium lactate and sodium diacetate (9, 16, 32, 33). Like lactates, sodium diacetate is a generally recognized as safe substance that is already used in foods for pH control, flavoring, and as an antimicrobial agent. The U.S. Food Safety and Inspection Service (FSIS) has amended the federal meat and poultry products inspection regulations to increase permissible levels of potassium lactate as a flavor enhancer in meat and poultry products, and of sodium diacetate as a flavor enhancer and as an inhibitor of the growth of certain pathogens. The final rule allows for the increase of lactates from 2 up to 4.8%, and the increase of sodium diacetate from 0.1 up to 0.25% (30). Studies indicate that sodium diacetate used at levels of 0.2% and higher suppresses the growth of *L. monocytogenes* (21, 24). However, sensory tests indicate that such high levels (0.2%) result in an unacceptable flavor in finished meat and poultry products (17). By using combinations of lactates with sodium diacetate at a maximum level of 0.12%, additional hurdles can be incorporated into producing unacceptable meat products. The synergistic effects of sodium lactate and sodium diacetate on inhibition of *L. monocytogenes* growth were observed in frankfurters, ham, and turkey products (3, 11, 20, 21). Blom et al. (3) already showed the advantage of using a combination of lactate and diacetate in sausage and cooked ham to inhibit the growth of *L. monocytogenes*. Recently, a predictive model to describe the effect of temperature, sodium lactate, and sodium diacetate on the inactivation of *L. monocytogenes* in frankfurter slurry has been introduced (22). Legan et al. (10) developed the model, which describes the growth boundary of *L. monocytogenes* in ready-to-eat cooked meat products at 4°C as a function of the product salt, moisture, potassium lactate, and sodium diacetate concentration, and validated the conditions to prevent the growth of *L. monocytogenes*, using various cured and uncured products. Vogel et al. (31) investigated the effect of combined potassium lactate and sodium diacetate on the sensory properties of vacuum-packed, cold-smoked salmon, and revealed that a combination of lactate and sodium diacetate does not negatively affect flavor or odor.

Yoon et al. (33) investigated the effect of pH and agitation on the growth of *L. monocytogenes* Scott A in brain heart infusion (BHI) broth containing a combination of potassium lactate and sodium diacetate during storage at 4 or 10°C. That study tested only one level of combination of lactate and diacetate (3.3%) on the growth rate of *L. monocytogenes* at only two temperatures, which was not enough to develop a model for the antimicrobial effect of a combination of potassium lactate and sodium diacetate on *L. monocytogenes* kinetics. In this study, we investigated the effect of a combination of potassium lactate and sodium diacetate (0, 1.8, 3, and 4.5%) on the survival and growth of *L. monocytogenes* in BHI broth adjusted at four different pHs (5.5, 6.0, 6.5, and 7.0) and incubated at six temperatures (4, 10, 17, 24, 30, and 37°C). This is the first set of static broth data reported in the literature where the inter-

action of lactate-diacetate concentration, pH, and temperature are detailed.

MATERIALS AND METHODS

Potassium lactate and sodium diacetate. A commercially prepared blend of potassium lactate and sodium diacetate (PURASAL P Opti.Form 4; potassium L-2-hydroxy-propionate and sodium hydrogen diacetate) was obtained from Purac America, Inc. (Lincolnshire, Ill.). PURASAL P Opti.Form 4 is a 60% natural, HiPure grade solution with a lactate-to-diacetate ratio of 14:1 (56% potassium lactate and 4% sodium diacetate).

Bacterial culture. A single strain of *L. monocytogenes* Scott A (ATCC 49594, American Type Culture Collection, Rockville, Md.) was maintained at -70°C at a concentration of 9.5 to 10 log CFU/ml in BHI broth (Difco, Becton Dickinson, Sparks, Md.) containing 15% glycerol. The stock culture was thawed at room temperature, and then 10 µl was inoculated into a 25-ml Erlenmeyer flask that contained 9 ml of sterile Trypticase soy broth with 0.6% yeast extract (Difco, Becton Dickinson). After inoculation, the culture was incubated on a rotary shaker (150 rpm) for 24 h at 37°C, under aerobic conditions. After incubation, 1 ml of the starter culture was serially diluted in 9 ml of 0.1% sterilized peptone water for inoculation into the BHI broths.

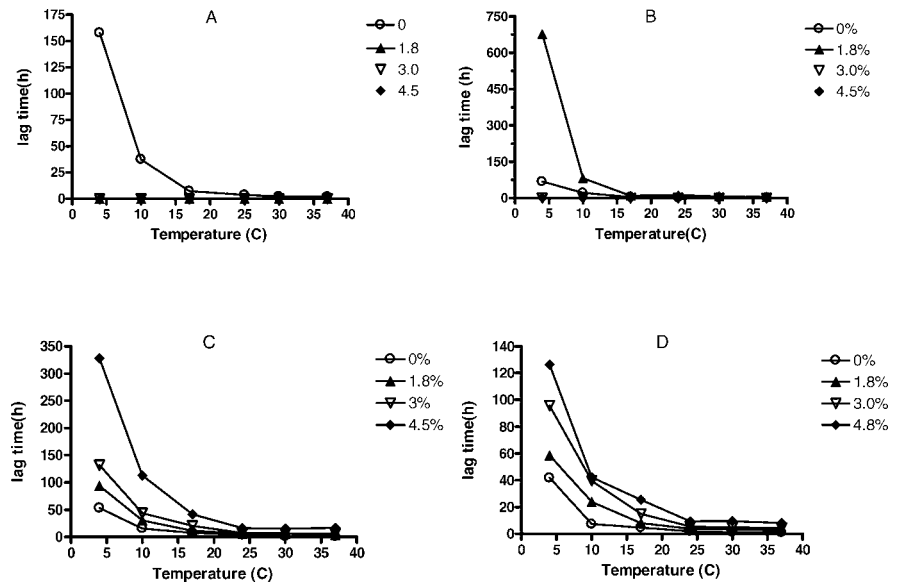
Preparation and inoculation of BHI broth. For each pH and temperature condition tested, 50 ml of BHI broth was prepared in four 250-ml Erlenmeyer flasks, followed by addition of 0 (control), 1.8 (1.68% lactate-0.12% diacetate), 3 (2.8% lactate-0.2% diacetate), or 4.5% (4.2% lactate-0.3% diacetate) of PURASAL P Opti.Form 4, respectively. The pH values of the BHI broths were then adjusted to 5.5, 6.0, 6.5, and 7.0, using sterile 1 M HCl and 1 M NaOH. The pH-adjusted broth was autoclaved at 121°C for 15 min. After cooling, flasks were stored overnight at the appropriate temperatures. The pH of each BHI broth was measured after autoclaving and readjusted, if necessary. On the following day, each flask was aseptically inoculated with 50 µl of a diluted culture of *L. monocytogenes*, to reach an initial population of 10^{2.5} CFU/ml. Flasks were immediately incubated at 4, 10, or 17°C (model 2005, Sheddon Manufacturing, Cornelius, Oreg.), or at 20, 24, 30, or 37°C (model 4230, New Brunswick Scientific, Edison, N.J.), without aeration.

Measurement of the pH. The pH was measured with an IQ 240 pH meter with a nonglass probe (IQ Scientific Instruments, Inc., San Diego, Calif.).

Colony count. At selected times postinoculation, depending on the incubation temperature and pH of the broth, 50-µl aliquots were taken, diluted as appropriate, spiral plated (Autoplate 4000, Spiral Biotech, Inc., Norwood, Mass.) onto tryptose agar plates (Difco, Becton Dickinson) in duplicate, and incubated at 37°C for 24 h. At each condition, sampling was conducted 8 to 12 times to obtain the growth curve. Colonies from each sample were counted with an automated colony counter (Q Count, Spiral Biotech), and the data were converted to log CFU per milliliter. All experiments were replicated three times.

The mean of the replicates was graphed at each sampling time to generate the growth data, which were iteratively fit to a three-phase linear model (4) using version 3.02 of Prism (GraphPad Software, San Diego, Calif.). During the lag phase, if $t \leq t_{LAG}$, then $N_t = N_0$; in the exponential growth phase, if $t_{LAG} < t < t_{MAX}$, then $N_t = N_0 + SGR(t - t_{LAG})$; and in the stationary phase, if $t \geq t_{MAX}$, then $N_t = N_{MAX}$, where N_t is the log of the population density at time t , N_0 is the log of the initial population

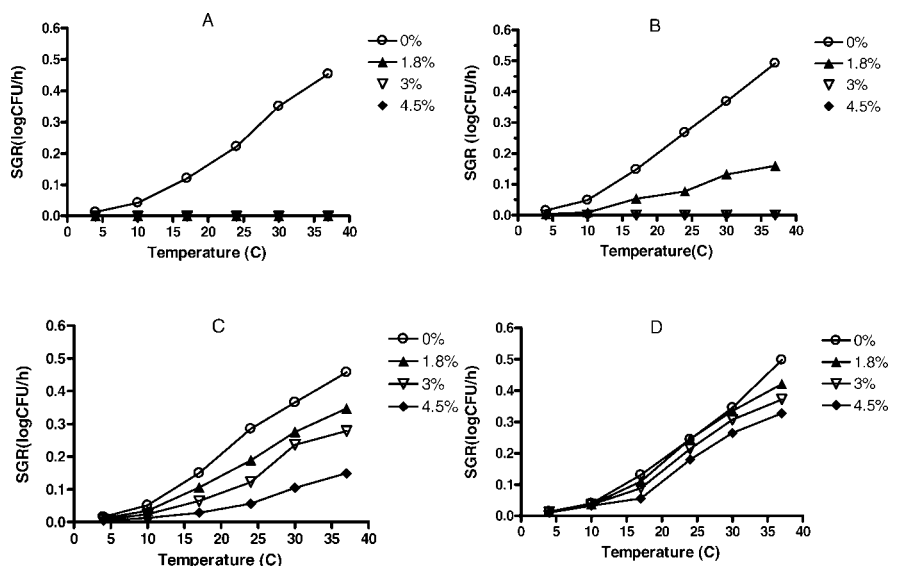
FIGURE 1. Effect of combinations of potassium lactate and sodium diacetate on the lag time of *L. monocytogenes* as a function of temperatures in pH-adjusted broth. A, pH 5.5; B, pH 6.0; C, pH 6.5; D, pH 7.0.



density, N_{MAX} is the log of the maximum population density supported by the environment, t is the elapsed time, t_{LAG} is the time when the lag phase ends (h), t_{MAX} is the time when the maximum population density is reached (h), and SGR is the specific growth rate ($\log CFU ml^{-1} h^{-1}$). Lag time (LT) and SGR were calculated for each growth curve. Since the inverse of the generation time is the growth rate, the generation time was determined from the growth rate. Relative lag time (RLT; the ratio of LT to generation time) was calculated and used to measure the amount of work to be done by the cell before growth is initiated in a new environment (13, 19).

Statistical analysis. All SGRs and LTs at each growth curve were analyzed using the General Linear Models procedure for analysis of variance (SPSS for Windows, version 11.5). To better assess the effect of the lactate-diacetate concentrations at different temperatures, the statistical analysis was conducted for each pH as an individual food formulation. The experiment was conducted using a two-way factorial arrangement (6×4) of six temperatures (4, 10, 17, 24, 30, and 37°C) and four concentrations of lactate-diacetate mixture (0, 1.8, 3, and 4.5%). Significant differences between the means of the treatments were determined using the least significant difference procedure at a significance level of 5%.

FIGURE 2. Effect of combinations of potassium lactate and sodium diacetate on the specific growth rate of *L. monocytogenes* as a function of temperatures in pH-adjusted broth. A, pH 5.5; B, pH 6.0; C, pH 6.5; D, pH 7.0.



RESULTS AND DISCUSSION

Effect of combinations of potassium lactate and sodium diacetate on LT and specific growth rate of *L. monocytogenes* Scott A in pH-adjusted broth as a function of temperature including, 4, 10, 17, 24, 30, and 37°C, are shown in Figures 1 and 2, respectively. The combination of potassium lactate and sodium diacetate used in this study (1.8, 3, and 4.5%) was lower than are the maximum levels allowed by the FSIS. However, the concentration of 4.5% contained 0.05% higher diacetate than the allowable level; this was done to evaluate the loss of activity of the dissociated diacetate anion around neutral pH. There were significant interactions between the four concentrations of lactate-diacetate mixture and storage temperatures over the four pH levels studied.

As shown in Figures 1A and 2A, pH 5.5 was the most restrictive growth condition involved in the present study. A listeristatic effect was observed for 1.8, 3, and 4.5% throughout the storage time at all tested temperatures. Listericidal effect was also observed with 4.5% concentration

at 4°C. On the other hand, the control sample reached maximum population density after 650 and 180 h at 4 and 10°C, respectively (data not shown). Duration of LT of the control sample was significantly ($P < 0.001$) affected by the storage temperature. Results of this study agree with those of Stekelenburg (27), which indicate almost full inhibition of *L. monocytogenes* growth during 29 days at 4°C in frankfurters prepared with a 2-to-3% solution of lactate-diacetate mixtures. A listeristatic effect of 1.8% sodium lactate and 0.1% sodium diacetate in meat at 5°C has also been reported by Mbandi and Shelef (12), and in turkey slurries by Schlyter et al. (21).

At pH 6.0, addition of 1.8% lactate-diacetate significantly ($P < 0.001$) increased LT up to 678 and 82 h of storage at 4 and 10°C, respectively (Fig. 1B). However, no significant effect was observed at 17, 24, 30, and 37°C. Addition of 1.8% lactate-diacetate also reduced significantly ($P < 0.001$) specific growth rate of *L. monocytogenes* compared with control, whereas 3% (2.8% lactate–0.2% diacetate) and 4.5% (4.2% lactate–0.3% diacetate) completely inhibited growth of *L. monocytogenes* at the six temperatures studied (Fig. 2B). Legan et al. (10) also observed no growth of *L. monocytogenes* in cured, vacuum-sealed Cotto salami, reduced-fat bologna, ham, and wieners, with 2.5% lactate and 0.15% diacetate at various salt-moisture combinations at 4°C.

At pH 6.5, 1.8% of the lactate-diacetate mixture had no significant effect on the growth rate of *L. monocytogenes* at the six temperatures studied (Fig. 2C), although it significantly increased LT at the lower temperatures, i.e., 4, 10, and 17°C (Fig. 1C). Overall, at least 3% was required to suppress growth rate of the pathogen at pH 6.5. Thus, it is recommended to add 3% of the mixture at this pH in order to achieve significant inhibition of *Listeria* growth. These findings support previous broth studies on *L. monocytogenes*, where addition of 3% of the lactate-diacetate mixture at pH 6.5 significantly controlled the growth of the pathogen at 4 and 10°C (33).

The results at pH 6.5 agreed with results reported by Samelis et al. (20), which indicated that the combination of 3% lactate-diacetate mixture reduced the growth of *L. monocytogenes* in frankfurters (pH 6.5) and bologna (pH 6.6) at 10°C. On the other hand, Barmpalia et al. (2) reported that 1.8% of the mixture was an effective treatment to control the growth of *L. monocytogenes* in pork bologna at both 4 and 10°C. The observed discrepancy regarding the effectiveness of these treatments could be explained by the change of pH value during the course of experiment for different food products and food formulations. In this study, addition of 1.8 and 3% of the lactate-diacetate mixture reduced the pH of the broth by 0.5 to 1.5 U before readjusting the pH. However, the pH values of the broth containing lactate-diacetate mixture remained within ± 0.05 U of the adjusted pH values during the time of storage throughout the trial, while the initial adjusted pH decreased from 6.5 to 6.2 and 7.0 to 6.8 for control. In contrast, Barmpalia et al. (2) reported a significant change of pH during storage of bologna at 4 and 10°C; the initial pH values of product formulated without antimicrobials was 6.57 to 6.61, while

treatments that caused significant pH reduction to 6.34 on day 0 were 1.8% sodium lactate combined with 0.25% sodium diacetate. Other authors observed a comparable reduction in the pH of meat when 0.25 to 0.5% diacetate was added to ground beef or beef slurry (25) and turkey slurries (21). The discrepancy of pH between the broth and food products might be attributed to the readjustment of the pH of the broth containing the mixtures to the desired pH.

As expected, a pH of 7.0 supported relatively rapid and prolific growth of *L. monocytogenes* (Figs. 1D and 2D). There was a significant ($P < 0.001$) effect of addition of lactate and diacetate mixture on the extension of LT at 4, 10, and 17°C (Fig. 1D). On the other hand, addition of 1.8 and 3% of lactate-diacetate mixture showed no statistically significant effects on the specific growth rate at the lower temperatures (4 and 10°C). However, a significant reduction of the specific growth rate was observed with addition of lactate and diacetate mixture at 30 and 37°C (Fig. 2D).

Maintaining homeostasis and repairing cellular damages often require several mechanisms to be activated, which is metabolically demanding for injured cells and thus, leads to an extended lag phase. Robinson et al. (19) hypothesized that lag could be determined by two parameters, the amount of work to be done to adapt to a new environment and the rate at which that work can be done. The ratio of LT divided by the generation time (RLT) is considered as a measure of the amount of work to be done by the cell before growth is initiated in that environment. In addition, RLT has been used to differentiate the effects of the osmotic (13) and temperature (14) shifts on the lag phase duration in the environment. Table 1 shows the effect of temperature and lactate-diacetate concentration on RLT of *L. monocytogenes* as a function of pH. In general, RLTs were increased as the environmental conditions became less favorable for growth of *L. monocytogenes*. Without lactate-diacetate, low pH and temperature were major factors that affected RLT. With 1.8 % of the lactate-diacetate, the highest RLT (3.775) was observed in the broth at pH 6 stored at 4°C. With addition of 3% lactate-diacetate, RLT values ranged from 1.259 to 2.644, indicating that lag is approximately proportional to or double the growth rate. RLT was further increased up to 3.793 with 4.5% of lactate-diacetate mixture at pH 7 and 37°C, indicating that lag is longer relative to growth rate at this condition. This result indicates that addition of 4.5% lactate-diacetate affects mainly LT and increases the amount of work to be done in a new environment.

In this study, we found that the combinations of potassium lactate and sodium diacetate were effective against growth of *L. monocytogenes* because of the strong synergistic effects of the mixture, especially at low pH. The antimicrobial activity of these chemicals was dependent on pH and to a lesser extent on temperature, especially, higher storage temperature.

In conclusion, the effectiveness of combinations of antimicrobials was assessed in this study at different concentrations (0 to 4.5%) of a lactate-diacetate mixture and over a wide range of storage temperatures (4 to 37°C). Indeed, 1.8% lactate-diacetate was adequate to control the growth

TABLE 1. Effect of temperature and lactate-diacetate concentration on relative lag time of *L. monocytogenes*

Temp	Opti.Form 4 (%)	Relative lag time ^a			
		pH 5.5	pH 6.0	pH 6.5	pH 7.0
4	0	2.914	1.480	1.211	0.839
	1.8	ND ^b	3.775	1.571	1.072
	3	ND	ND	1.503	1.683
	4.5	ND	ND	2.13	1.986
10	0	2.299	1.505	1.153	0.415
	1.8	ND	1.047	1.532	1.259
	3	ND	ND	1.536	2.087
	4.5	ND	ND	2.085	1.966
17	0	1.266	0.926	1.582	0.882
	1.8	ND	0.746	1.777	1.290
	3	ND	ND	1.918	1.899
	4.5	ND	ND	1.722	2.054
24	0	1.209	0.685	1.664	0.735
	1.8	ND	1.337	1.8	1.346
	3	ND	ND	1.27	1.695
	4.5	ND	ND	1.327	2.417
30	0	1.141	0.775	0.689	0.684
	1.8	ND	1.378	2.377	1.601
	3	ND	ND	2.388	2.124
	4.5	ND	ND	2.316	3.625
37	0	1.357	1.203	0.875	0.827
	1.8	ND	1.260	2.021	1.756
	3	ND	ND	2.644	2.322
	4.5	ND	ND	3.584	3.793

^a The ratio of the lag time divided by the generation time.

^b ND, not determined.

of *L. monocytogenes* at pH 5.5. However, 1.8 and 3% of the lactate-diacetate mixture were able to extend the lag phase at refrigeration temperature, regardless of pH. As the pH of different food products after addition of 1.8% lactate-diacetate mixture ranges between 5.5 and 6.5, the data suggest that 1.8% may be used to control growth of *L. monocytogenes*, thus enhancing the margins of safety for refrigerated meat products. It is concluded that 1.8% lactate-diacetate mixture can be used as a substantial hurdle to control the growth of *L. monocytogenes* for the cultural conditions described above. These data will be used to develop a predictive model for growth of *L. monocytogenes* in broth as a function of lactate and diacetate concentration, pH, and temperature.

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REFERENCES

- Aran, N. 2001. The effect of calcium and sodium lactates on growth from spores of *Bacillus cereus* and *Clostridium perfringens* in a "sous-vide" beef goulash under temperature abuse. *Int. J. Food Microbiol.* 63:117-123.
- Barmpalia, I. M., K. P. Koutsoumanis, I. Geornaras, K. E. Belk, J. A. Scangaa, P. A. Kendall, G. C. Smith, and J. N. Sofos. 2005. Effect of antimicrobials as ingredients of pork bologna for *Listeria monocytogenes* control during storage at 4 or 10°C. *Food Microbiol.* 22: 205-211.
- Blom, H., E. Nerbrink, R. Dainty, T. Hagtvedt, E. Borch, H. Nissen, and T. Nesbakken. 1997. Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum packed, sensory-acceptable serelat sausage and cooked ham stored at 4°C. *Int. J. Food Microbiol.* 38:71-76.
- Buchanan, R. L., R. C. Whiting, and W. C. Damert. 1997. When simple is good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiol.* 14:313-326.
- De Wit, J. C., and F. M. Rombouts. 1990. Antimicrobial activity of sodium lactate. *Food Microbiol.* 7:113-120.
- Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55: 476-511.
- Glass, K. A., D. A. Granberg, A. L. Smith, A. M. McNamara, M. Hardin, J. Mattias, K. Ladwig, and E. A. Johnson. 2002. Inhibition of *Listeria monocytogenes* by sodium diacetate and sodium lactate on wieners and cooked bratwurst. *J. Food Prot.* 65:116-123.
- Golden, M. H., R. L. Buchanan, and R. C. Whiting. 1995. Effect of sodium acetate or sodium propionate with EDTA and ascorbic acid on the inactivation of *Listeria monocytogenes*. *J. Food Saf.* 15:53-65.
- Juneja, V. K. 2003. Predictive model for the combined effect of temperature, sodium lactate, and sodium diacetate on the heat resistance of *Listeria monocytogenes* in beef. *J. Food Prot.* 66:804-811.
- Legan, J. D., D. L. Seman, A. L. Milkowski, J. A. Hirschey, and M. H. Vandeven. 2004. Modeling the growth boundary of *Listeria monocytogenes* in ready-to-eat cooked meat products as a function of the product salt, moisture, potassium lactate, and sodium diacetate concentrations. *J. Food Prot.* 67:2195-2204.
- Lu, Z., J. G. Sebranek, J. S. Dickson, A. F. Mendonca, and T. B. Bailey. 2005. Inhibitory effects of organic acid salts for control of *Listeria monocytogenes* on frankfurters. *J. Food Prot.* 68:499-506.
- Mbandi, E., and L. A. Shelof. 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* in meat by combinations of sodium lactate and diacetate. *J. Food Prot.* 64:640-644.

13. Mellefont, L. A., T. A. McMeekin, and T. Ross T. 2003. The effect of abrupt osmotic shifts on the lag phase duration of foodborne bacteria. *Int. J. Food Microbiol.* 83:281–29.
14. Mellefont, L. A., and T. Ross. 2003. The effect of abrupt temperature shifts on the lag phase duration of *Escherichia coli* and *Klebsiella oxytoca*. *Int. J. Food Microbiol.* 83:295–305.
15. Miller, R. K., and G. R. Acuff. 1994. Sodium lactate affects pathogens in cooked beef. *J. Food Sci.* 59:15–19.
16. Nerbrink, E., E. Borch, H. Blom, and T. Nesbakken. 1999. A model based on absorbance data on the growth of *Listeria monocytogenes* and including the effects pH, NaCl, Na-lactate and Na-acetate. *Int. J. Food Microbiol.* 47:99–109.
17. Purac America, Inc. 2001. Purac Opti.Form® *Listeria* control model information sheet. Available at: http://www.purac.com/purac_com/fe062d8ce71f4d67cee300183be4de36.php.
18. Qvist, S., K. Sehested, and P. Zeuthen. 1994. Growth suppression of *Listeria monocytogenes* in a meat product. *Int. J. Food Microbiol.* 24:283–293.
19. Robinson, T. P., M. J. Ocio, A. Kaloti, and M. M. Bernard. 1998. The effect of the growth environment on the lag phase of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 44:83–92.
20. Samelis, J., G. K. Bedie, J. N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 10°C in vacuum packages. *J. Food Prot.* 65:299–307.
21. Schlyter, J. H., K. A. Glass, J. Loefelholz, A. J. Degnan, and J. B. Luchansky. 1993. The effect of diacetate with nitrite, lactate, or pediocin on viability of *Listeria monocytogenes* in turkey slurries. *Int. J. Food Microbiol.* 19:271–281.
22. Schultze, K. K., R. H. Linton, M. A. Cousin, J. B. Luchansky, and M. L. Tamplin. 2006. A predictive model to describe the effects of temperature, sodium lactate, and sodium diacetate on the inactivation of serotype 4b strain of *Listeria monocytogenes* in frankfurter slurry. *J. Food Prot.* 69:1552–1560.
23. Shelef, L. A. 1994. Antimicrobial effects of lactate: a review. *J. Food Prot.* 57:445–450.
24. Shelef, L.A., and L. Addala. 1994. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. *J. Food Saf.* 14:103–115.
25. Shelef, L. A., and Q. Yang. 1991. Growth suppression of *Listeria monocytogenes* by lactates in broth, chicken, and beef. *J. Food Prot.* 54:283–287.
26. Sofos, J. N. 1993. Current microbiological considerations in food preservation. *Int. J. Food Microbiol.* 19:87–108.
27. Stekelenburg, F. K. 2003. Enhanced inhibition of *Listeria monocytogenes* in frankfurter sausage by the addition of potassium lactate and sodium diacetate mixtures. *Food Microbiol.* 20:133–137.
28. Stekelenburg, F. K., and M. L. T. Kant-Muermans. 2001. Effects of sodium lactate and other additives in a cooked ham product on sensory quality and development of a strain of *Lactobacillus curvatus* and *Listeria monocytogenes*. *Int. J. Food Microbiol.* 66:197–203.
29. U.S. Food and Drug Administration. 2001. Processing parameters needed to control pathogen in cold-smoked fish. *J. Food Sci.* 66(Suppl.):1055–1134.
30. U.S. Food Safety and Inspection Service. 2000. FSIS to increase permissible levels of food ingredients used as antimicrobials and flavoring agents. *Fed. Regist.* 65:3121–3123.
31. Vogel, B. F., Y. Yin Ng, G. Hyldig, M. Mohr, and L. Gram. 2006. Potassium lactate combined with sodium diacetate can inhibit growth of *Listeria monocytogenes* in vacuum-packed cold-smoked salmon and has no adverse sensory effects. *J. Food Prot.* 69:2134–2142.
32. Yoon, K. S., C. N. Burnette, K. A. Abou-Zeid, and R. C. Whiting. 2004. Control of growth and survival of *Listeria monocytogenes* on smoked salmon by combined potassium lactate and sodium diacetate and freezing stress during refrigeration and frozen storage. *J. Food Prot.* 67:2465–2471.
33. Yoon, K. S., C. N. Burnette, and R. C. Whiting. 2003. Effects of pH and agitation on the growth of *Listeria monocytogenes* in broth containing combined potassium lactate and sodium diacetate during storage at 4 or 10°C. *J. Food Prot.* 66:1469–1473.