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Modeling the Growth of *Listeria monocytogenes* in Cured Ready-to-Eat Processed Meat Products by Manipulation of Sodium Chloride, Sodium Diacetate, Potassium Lactate, and Product Moisture Content

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ABSTRACT

A central composite second-order response surface design was employed to determine the influences of added sodium chloride (0.8 to 3.6%), sodium diacetate (0 to 0.2%), potassium lactate syrup (0.25 to 9.25%), and finished-product moisture (45.5 to 83.5%) on the predicted growth rate of *Listeria monocytogenes* in cured ready-to-eat (RTE) meat products. Increased amounts of both sodium diacetate ($P < 0.11$) and potassium lactate ($P < 0.001$) resulted in significant reductions in the growth rate constants of *L. monocytogenes.* Increased finished-product moisture ($P < 0.11$) significantly increased growth rate constants. The influence of sodium chloride was not statistically significant. The second-order statistical factor for lactate was significant ($P < 0.01$), but all two-way interactions were not. In general, predicted growth rates exceeded actual growth rates obtained from inoculation studies of four cured RTE meat products (wieners, smoked-cooked ham, light bologna, and cotto salami). The final model will be useful to food technologists in determining formulations that will result in finished cured RTE meat products in which *L. monocytogenes* is not likely to grow.

Listeria monocytogenes has become a significant foodborne pathogen in recent years because of its ability to cause foodborne infections that can lead to headache, fever, chills, and diarrhea; severe cases can lead to septicemia, meningitis, and abortion. Approximately 20 to 30% of human listeriosis cases result in death for susceptible individuals *(14, 17, 21).* An outbreak of listeriosis in 1998 that caused approximately 100 illnesses and 21 deaths was traced to the postprocess contamination of packaged wieners at a large commercial plant *(8).* Although the risk of severe listeriosis exists for susceptible individuals, the occurrence of *L. monocytogenes* in processed meat products is relatively low. The Food Safety Inspection Service *(2)* recently released a survey that reported that the incidence of *L. monocytogenes* in small-diameter sausages was 1.8 to 4.1% of samples taken between 1993 and 1999. The incidence of *L. monocytogenes* in sliced ham and pork was found to be 4.2 to 5.7% of samples taken during the same period. However, because of the high mortality rate of listeriosis, formulation-based and other processing-based measures to retard or prevent the potential growth of *L. monocytogenes* in refrigerated cured ready-to-eat (RTE) meat products should be considered. These measures include irradiation *(11, 25),* ultra-high-pressure processing *(16),* postpackaging pasteurization, and/or the addition of antimicrobial compounds.

The properties of a number of known natural bactericidal compounds have been studied and summarized *(24).* These compounds include organic acids, bacteriocins, phenolic compounds, plant extracts, and synthetic peptides *(4, 5, 7, 15).* Two compounds showing promising results are acetate and lactate and their derivatives *(6, 18).* Combinations of lactate and diacetate have also been shown to exhibit anti-listerial activity *(20).* Blom et al. *(6)* found that the combination of 2.5% lactate and 0.25% acetate prevented the growth of *L. monocytogenes* in servelat sausage while maintaining the sensory acceptability of the sausage. Thus, the addition of sufficient amounts of lactate and diacetate can result in an RTE meat product that will not support the growth of *L. monocytogenes.*

A model allowing the estimation of sodium diacetate and lactate amounts that are sufficient to provide a barrier for potential *L. monocytogenes* growth would be of great value to manufacturers of RTE meat products. This technique should encompass wide ranges of finished-product variables, including product moisture and NaCl content, since the amount of diacetate and lactate needed may change with fluctuations of these variables. Product developers could then use this model to modify product formulas by adding specific amounts of lactate salts and sodium diacetate to limit the growth of *L. monocytogenes.*

This study was designed to predict the growth of *L. monocytogenes* using a response surface method (RSM) for cured RTE meat products made with a wide range of NaCl, sodium diacetate, potassium lactate, and product moisture contents that are commonly observed in cured meat prod-

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TABLE 1. *Design matrix of the central composite design for modeling the growth of* L. monocytogenes *in cured meats*

Run	Salt[a]	Sodium diacetate[a]	Potassium lactate syrup[a]	Product moisture[a]	% salt[b]	% sodium diacetate[b]	% potassium lactate syrup[b,c]	% product moisture[b]
1	1	−1	−1	−1	2.90	0.05	2.5	55.0
2	−1	1	−1	−1	1.50	0.15	2.5	55.0
3	−1	−1	1	−1	1.50	0.05	7.0	55.0
4	1	1	1	−1	2.90	0.15	7.0	55.0
5	−1	−1	−1	1	1.50	0.05	2.5	74.0
6	1	1	−1	1	2.90	0.15	2.5	74.0
7	1	−1	1	1	2.90	0.05	7.0	74.0
8	−1	1	1	1	1.50	0.15	7.0	74.0
9	0	0	0	0	2.20	0.10	4.75	64.5
10	0	0	0	0	2.20	0.10	4.75	64.5
11	−1	−1	−1	−1	1.50	0.05	2.5	55.0
12	1	1	−1	−1	2.90	0.15	2.5	55.0
13	1	−1	1	−1	2.90	0.05	7.0	55.0
14	−1	1	1	−1	1.50	0.15	7.0	55.0
15	1	−1	−1	1	2.90	0.05	2.5	74.0
16	−1	1	−1	1	1.50	0.15	2.5	74.0
17	−1	−1	1	1	1.50	0.05	7.0	74.0
18	1	1	1	1	2.90	0.15	7.0	74.0
19	0	0	0	0	2.20	0.10	4.75	64.5
20	0	0	0	0	2.20	0.10	4.75	64.5
21	−2	0	0	0	0.80	0.10	4.75	64.5
22	2	0	0	0	3.60	0.10	4.75	64.5
23	0	−2	0	0	2.20	0	4.75	64.5
24	0	2	0	0	2.20	0.20	4.75	64.5
25	0	0	−2	0	2.20	0.10	0.25	64.5
26	0	0	2	0	2.20	0.10	9.25	64.5
27	0	0	0	−2	2.20	0.10	4.75	45.5
28	0	0	0	2	2.20	0.10	4.75	83.5
29	0	0	0	0	2.20	0.10	4.75	64.5
30	0	0	0	0	2.20	0.10	4.75	64.5

[a] Statistical design coefficients.
[b] Ingredients were added as percentages of total formula weight. Finished-product moisture was calculated with a linear computer program (What's Best!, Lindo Systems).
[c] Potassium lactate syrup contained 60% lactate by weight.

ucts. The data from the individual treatments were used in the development of a simple mathematical model capable of predicting the growth or stasis of *L. monocytogenes* in commercial cured meat products. More complex sigmoid growth models do exist (modified Gompertz and logistic models *(13)*) that can more precisely model bacterial growth in broth systems; however, they are not well suited to modeling bacterial growth in applied product experiments, since the products are not usually sampled at sufficient time intervals. In some instances, growth is immediate and no lag time is noted. In situations in which growth is diminished, the lag time is infinite. This simpler kinetic model may lose some precision in characterizing microbial growth rates in some instances, but in this applied situation, it seemed to be more appropriate. This model can facilitate the determination of the appropriate amounts of potassium lactate and sodium diacetate to add to a wide variety of cured meat products to obtain a sensory-acceptable product that will not support the growth of *L. monocytogenes.*

MATERIALS AND METHODS

Sample preparation. A central composite design, complete with star points and six replicates of the center point, was used for this study. The factors and levels used are reported in Table 1. The 30 products (runs) required for the experiment were formulated by using a variety of raw material sources, including pork trimmings (42% lean), trimmed turkey breast halves, and four-muscle ham (including the semimembranosus, semitendinosus, adductor, and gluteus medius muscles) purchased from commercial sources. All meats were ground at 0.634 cm immediately prior to use. Each product also contained sodium erythrobate (317 ppm), modified starch (1%; Firmtex, National Starch, Bridgewater, N.J.), sodium nitrite (97 ppm), carrageenan (0.35%; Gelcarin PS 3262, FMC Corporation, Princeton, N.J.), sodium tripolyphosphate (0.276%), and water (amounts varied depending on the desired finished-product moisture content). Potassium lactate (Purasal P/HQ60, PURAC America, Blair, Nebr.), and sodium diacetate (Macco Organiques, Inc., Valleyfield, Quebec, Canada) were used in various amounts according to the protocol.

The 30 separate products were formulated with a linear pro-

TABLE 2. *Growth rates of* L. monocytogenes *obtained from this study*

Run	% salt[a]	% sodium diacetate[a]	% potassium lactate syrup[a,b]	% product moisture[a]	*L. monocytogenes* growth rate (wk^{-1})
1	2.90	0.05	2.5	55.0	0.0035
2	1.50	0.15	2.5	55.0	0
3	1.50	0.05	7.0	55.0	0.0024
4	2.90	0.15	7.0	55.0	0
5	1.50	0.05	2.5	74.0	0.0991
6	2.90	0.15	2.5	74.0	0.0069
7	2.90	0.05	7.0	74.0	0
8	1.50	0.15	7.0	74.0	0
9	2.20	0.10	4.75	64.5	0
10	2.20	0.10	4.75	64.5	0
11	1.50	0.05	2.5	55.0	0.018
12	2.90	0.15	2.5	55.0	0
13	2.90	0.05	7.0	55.0	0
14	1.50	0.15	7.0	55.0	0
15	2.90	0.05	2.5	74.0	0.023
16	1.50	0.15	2.5	74.0	0
17	1.50	0.05	7.0	74.0	0
18	2.90	0.15	7.0	74.0	0
19	2.20	0.10	4.75	64.5	0
20	2.20	0.10	4.75	64.5	0
21	0.80	0.10	4.75	64.5	0.0069
22	3.60	0.10	4.75	64.5	0
23	2.20	0	4.75	64.5	0.0126
24	2.20	0.20	4.75	64.5	0
25	2.20	0.10	0.25	64.5	0.1338
26	2.20	0.10	9.25	64.5	0
27	2.20	0.10	4.75	45.5	0
28	2.20	0.10	4.75	83.5	0.032
29	2.20	0.10	4.75	64.5	0
30	2.20	0.10	4.75	64.5	0

[a] Ingredients were added as percentages of total formula weight. Finished-product moisture was calculated with a linear computer program (What's Best!, Lindo Systems).
[b] Potassium lactate syrup contained 60% lactate by weight.

TABLE 3. *Analysis of variance for growth rate constants of* L. monocytogenes[a]

Source	df	Sum of squares	*F* ratio	Probability
A: salt	1	0.00041583	1.0	0.33
B: diacetate	1	0.00112477	2.7	0.11
C: potassium lactate syrup	1	0.00720027	17.2	0.0004
D: product moisture	1	0.00119145	2.8	0.105
CC[b]	1	0.00605346	14.4	0.0009
Total error	24	0.0100738		

[a] The model was condensed by eliminating all nonsignificant ($P > 0.05$) two-way interactions and second-order terms. $r^2 = 0.61$; standard error of estimate = 0.02. Lack of fit could not be tested because all center points in the data set had growth rate constants of zero.
[b] CC denotes a second-order source of lactate syrup.

TABLE 4. *Regression coefficients for predicting the growth of* L. monocytogenes *in cured meat products*

Factor	Regression coefficient
Constant	0.07979
A: salt	−0.00595
B: diacetate	−0.13692
C: potassium lactate syrup	−0.03490
D: product moisture	0.00074
CC[a]	0.00286

[a] CC denotes a second-order term for lactate syrup.

gram (What's Best!, Lindo Systems, Inc., Chicago, Ill.) to determine the proper amount of water to include to obtain the formulation set points shown in Table 1. These products required mixtures of the various meats listed above. All meats, all dry ingredients, and water were blended under vacuum for 30 min at 45 rpm with a 220-kg Keebler mixer (Keebler Engineering, Inc., Chicago, Ill.). The meat batters were subsequently stuffed into nonpermeable casings (4.6 cm in diameter) and cooked in water tanks for 1 h at 49°C, 1 h at 60°C, and ca. 2 h at 85°C until the products reached an internal temperature of 74°C. The products were chilled in 4°C water and subsequently chilled in a 0°C cooler so that the internal temperature of the products was <4°C within 8 h of cooking.

After chilling, the products were stripped of their casings and sliced into 25-g slices; four slices were placed into pouches (Curlon Grade 863, nylon structure with polyethylene seal and PVDC barrier, oxygen transmission rate <1.0 cm^3/645 cm^2/24 h at 23°C and 0% relative humidity, moisture vapor transmission rate <0.5 g/645 cm^2/24 h at 38°C and 90% relative humidity; Curwood, Oshkosh, Wis.).

Samples used for validation of the model consisted of four varieties of cured meat products (wieners, ham, light bologna, and cotto salami) manufactured using three different formulations of potassium lactate and sodium diacetate. A formulation of 0% potassium lactate solids and 0% sodium diacetate was used for treatment 1, a formulation of 1.5% potassium lactate solids and 0.15% sodium diacetate was used for treatment 2, and a formulation of 2.5% potassium lactate solids and 0.15% sodium diacetate was used for treatment 3.

TABLE 5. *Performance factors calculated for the control validation products containing no diacetate or lactate*[a]

Treatment	Predicted growth rate (wk^{-1})	Observed growth rate (wk^{-1})	Log(predicted/observed)	Absolute value
Light bologna	0.112	0.182	−0.21	0.21
Wieners	0.108	0.033	0.51	0.51
Smoked/cooked ham	0.121	0.067	0.26	0.26
Cotto salami	0.113	0.176	−0.19	0.19
Mean			0.09	0.29
Bias factor[b]			1.24	
Accuracy factor[c]				1.97

[a] Ross *(19)*.
[b] $Antilog_{10}$ 0.09.
[c] $Antilog_{10}$ 0.29.

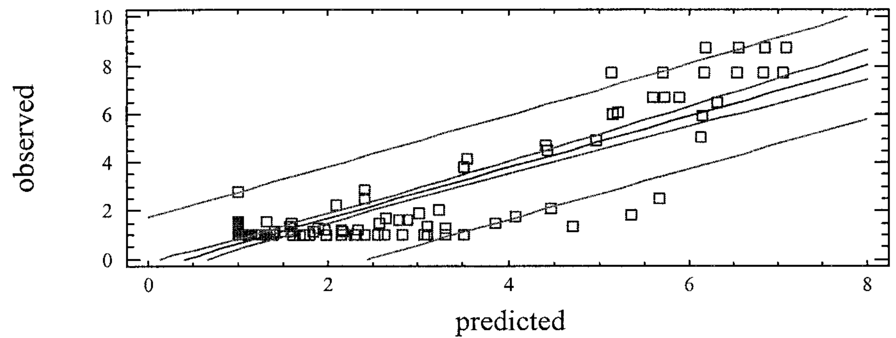

FIGURE 1. *Simple regression of observed versus predicted* L. monocytogenes *counts for all of the data derived in the study.* $r^2 = 76.4$*; standard error of estimate = 1.08* log_{10} *CFU/g. Inner lines represent the 95% confidence limit for the mean; outer lines represent the 95% confidence limit for prediction.*

Chemical analysis. Samples for each treatment were submitted for analysis of moisture, protein, fat, and ash; these analyses were conducted using AOAC methods 950.46, 992.15, 991.36, and 920.153, respectively *(3)*. NaCl was assayed as chloride ions by a titrimetric method *(1)*. Sodium lactate and sodium diacetate were analyzed by blending 5 g of ground meat with 30 ml of distilled water using a polytron for 30 s; the mixture was subsequently centrifuged (20 min at 2,500 rpm) and filtered through Whatman no. 1 filter paper into a clean 50-ml disposable centrifuge tube. An aliquot of filtrate was subsequently filtered a second time with a 0.2-μm membrane filter and transferred to an autoinjector vial. The filtrate was analyzed directly by using a high-performance liquid chromatograph (Perkin Elmer Series 200) with a flow rate of 0.6 ml/min, an injection of 20 μl, a 220-nm detector, and a run time of 20 to 30 min. The sodium lactate content was converted to potassium lactate with the appropriate conversion factor.

Microbiological methods: bacterial strains and growth conditions. Three *L. monocytogenes* isolates from foodborne outbreaks (LCDC 861, F2399, and NFPA 83) and two environmental isolates (MAD 225 and MAD 328) were used in a five-strain cocktail throughout this study. Strains were grown aerobically (without shaking) in 10 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) for 24 h at 25°C, allowing the cultures to reach the late stationary phase. Culture (0.1 ml) was serially transferred to 10 ml of fresh brain heart infusion broth and the incubation was repeated.

Microbiological methods: inoculum preparation and procedure. The inoculum was prepared by transferring 0.2 ml of each strain into 99 ml of Butterfield's phosphate buffer (BPB; Weber Scientific, Hamilton, N.J.). Serial dilutions were made in BPB to achieve the desired inoculum level. For RSM samples, the desired inoculum level was 1,000 CFU/g. The inoculum (100 μl total) was applied to the surface of 100 g of cured meat (four slices). Three 25-μl aliquots were placed between slices, and one was placed on the surface of the top slice. For validation studies, the desired inoculum level was 1 to 100 CFU/g. The inoculum (100 μl total) was applied to the surface of 100 g of cured meat (four slices or four links) in the manner described above for sliced meats, with one 25-μl deposit per link for wieners. All RSM samples were inoculated with the same inoculum on the same day; all validation samples were prepared and inoculated on separate days. The pouches containing the meat were immediately vacuum-sealed (Multivac C1400) and stored at 4°C for up to 18 weeks. The inoculum level was determined by enumerating the BPB on modified Oxford (MOX) agar spread plates (Oxoid, Ontario, Canada) and dividing the CFU per milliliter by 100 g.

Evaluation and enumeration of *L. monocytogenes*: RSM samples. Three samples of each treatment were analyzed every other week for *L. monocytogenes* by appropriately diluting the samples in BPB, direct plating them onto MOX agar, and incubating the plates for 48 h at 30°C. Colonies producing a black precipitate, indicative of *Listeria* spp., were considered *L. monocytogenes* colonies.

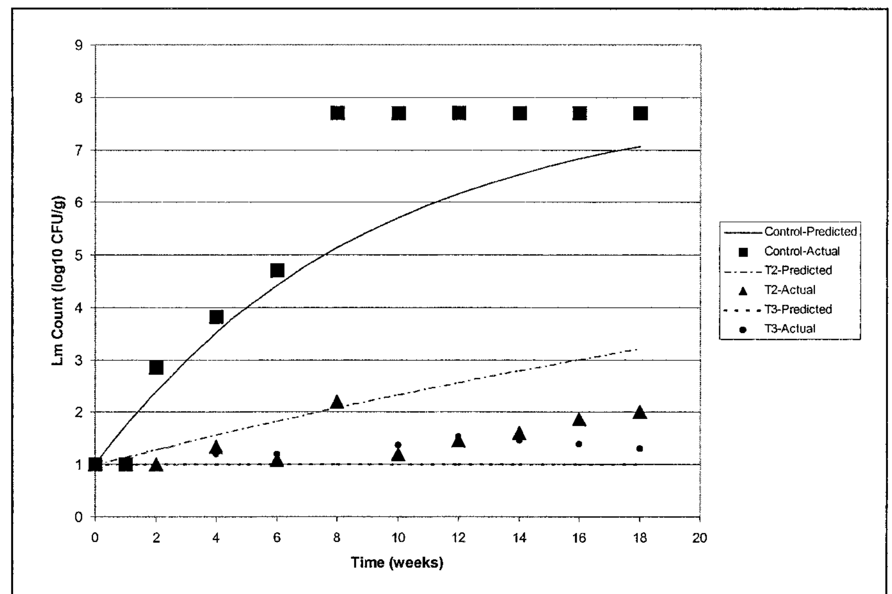

FIGURE 2. *Plot of* L. monocytogenes *growth on slices of light bologna inoculated with a five-strain* L. monocytogenes *cocktail over 18 weeks of storage at 4°C. Controls contain no potassium lactate or sodium diacetate. T2 contains 1.5% potassium lactate solids and 0.15% sodium diacetate. T3 contains 2.5% potassium lactate solids and 0.15% sodium diacetate.*

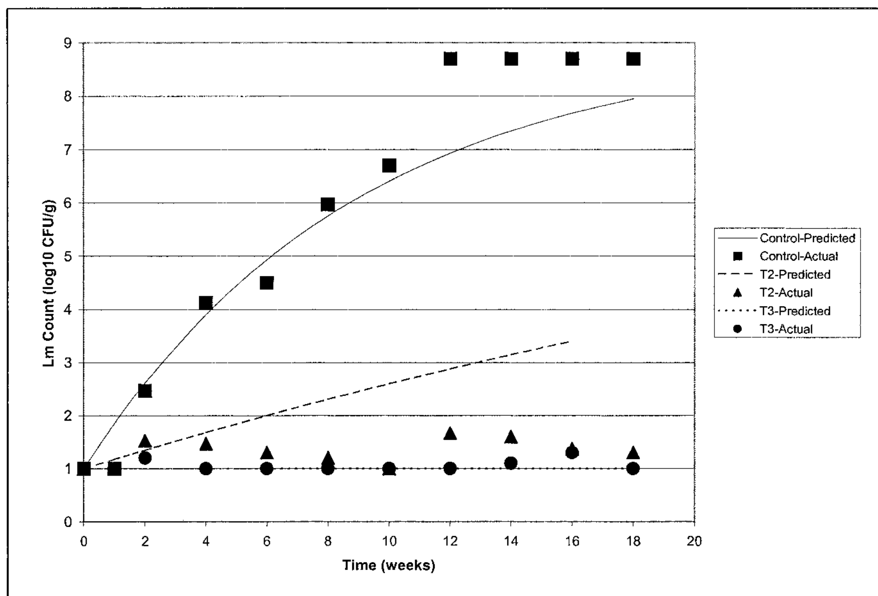

FIGURE 3. *Plot of* L. monocytogenes *growth on slices of cotto salami inoculated with a five-strain* L. monocytogenes *cocktail over 18 weeks of storage at 4°C. Controls contain no potassium lactate or sodium diacetate. T2 contains 1.5% potassium lactate solids and 0.15% sodium diacetate. T3 contains 2.5% potassium lactate solids and 0.15% sodium diacetate.*

Evaluation and enumeration of *L. monocytogenes*: validation samples. Three samples for each treatment were analyzed biweekly for *L. monocytogenes* as described above with the following exception: the large sample size required a dilution in BPB of 1:2 and then direct plating onto 0.1 ml of MOX agar, resulting in a minimum detection limit of 20 CFU/g. In the event that a sample yielded no colonies on a selective agar plate but did test positive for the presence of viable *L. monocytogenes,* the sample was recorded as positive by a modified U.S. Department of Agriculture cultural enrichment method (see below), but it was recorded as $\log_{10}$ 1.3 (20 CFU/g) for calculation purposes.

A modified U.S. Department of Agriculture cultural method was employed to determine the presence of *L. monocytogenes* below the minimum detection limit of MOX agar (20 CFU/g). All of the contents of the sample product (100 g) were added to 500 ml of UVM modified listeria enrichment broth (Difco) and incubated for 24 h at 30°C. A 0.1-ml portion of this primary enrichment was transferred to 10 ml of Fraser broth (Difco) containing 0.1 ml of Fraser broth supplement (Difco) and incubated for 24 to 48 h at 35°C. A loop of this secondary enrichment was then streaked onto MOX agar and incubated for 24 to 48 h at 35°C. Colonies producing a black precipitate, indicative of *Listeria* spp., were considered positive for *L. monocytogenes.*

Experimental design and data analysis. A rotatable cube central composite RSM design was used for this four-factor experiment. The design consisted of 16 factorial treatments augmented with eight star points and six center points, for a total of 30 treatments (Table 1). The radius for the star points was calculated as $2^{n/4}$, where n is the number of variables in the model *(9).* The response variable, the *L. monocytogenes* growth rate constant (k), was estimated for each treatment with nonlinear regression (Marquardt algorithm) and the simple kinetic equation

$$L.\ monocytogenes\ \text{count} = \text{Initial count} + \text{Span}(1 - e^{-kt})$$

where Initial count is the number of inoculated organisms ($\log_{10}$ CFU/g), Span is the span in counts from the initial count to the maximum count observed ($\log_{10}$ CFU/g), k is the growth rate constant (per week), and t is time (in weeks). In this study, the initial *L. monocytogenes* count was 4.0 $\log_{10}$ CFU/g and the max-

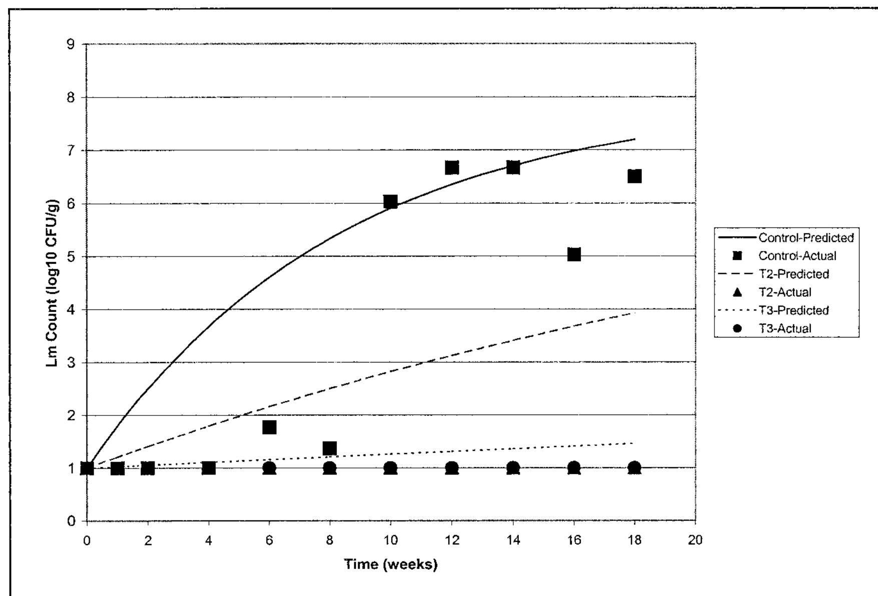

FIGURE 4. *Plot of* L. monocytogenes *growth on slices of smoked-cooked ham inoculated with a five-strain* L. monocytogenes *cocktail over 18 weeks of storage at 4°C. Controls contain no potassium lactate or sodium diacetate. T2 contains 1.5% potassium lactate solids and 0.15% sodium diacetate. T3 contains 2.5% potassium lactate solids and 0.15% sodium diacetate.*

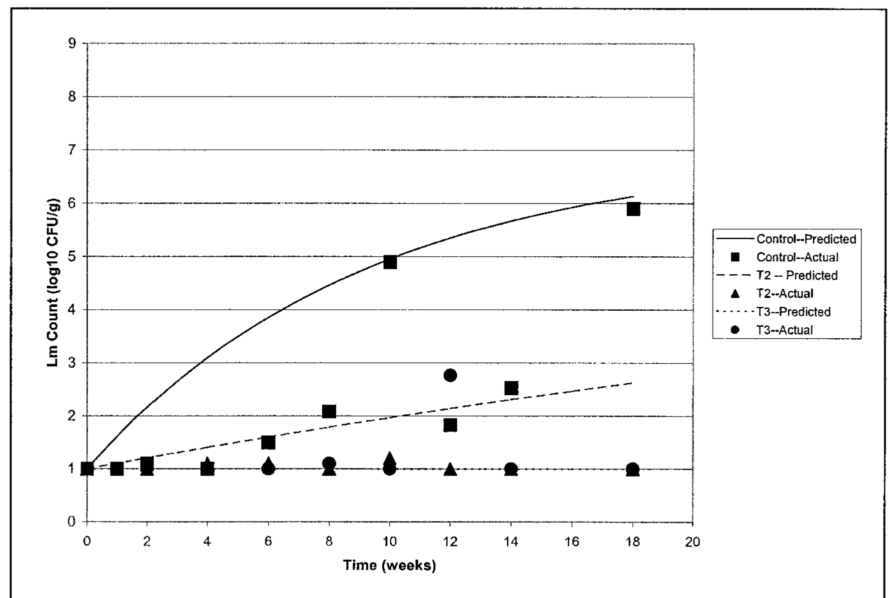

FIGURE 5. *Plot of* L. monocytogenes *growth on wieners inoculated with a five-strain* L. monocytogenes *cocktail over 18 weeks of storage at 4°C. Controls contain no potassium lactate or sodium diacetate. T2 contains 1.5% potassium lactate solids and 0.15% sodium diacetate. T3 contains 2.5% potassium lactate solids and 0.15% sodium diacetate.*

imum observed count was 8 $\log_{10}$ CFU/g; thus, the span was set to 4 $\log_{10}$ CFU/g. This treatment of the data also simplified the analysis and allowed a practical application of the model. Since this model was used only to model *L. monocytogenes* growth, any negative growth rate constants were set to 0 for the analysis of RSM data. Both the calculation of the growth rate constants and the statistical analysis of the central composite design were carried out with STATGRAPHICS*Plus,* version 4 (Manugistics, Inc., Rockville, Md.). All main effects (salt, sodium diacetate, potassium lactate, and product moisture) were left in the model even if $P > 0.10$, since they were the factors of concern at the outset of the experiment. Insignificant ($P > 0.10$) two-way interactions and second-order terms were removed from the model. The final model represented the influence of the main effects and significant second-order terms. Lag time was not considered in this model primarily because sampling (every 2 weeks) was not frequent enough to define a precise lag time. Omitting lag time provided a more simplified model that we deemed more useful than one including lag time. This simple kinetic model was chosen over others, including some sigmoid growth models (such as the logistic and modified Gompertz models) commonly used by food microbiologists, for several reasons, including consideration of the food product being tested, the logistics of the experiment, and the desire to use the results to aid in the formulation of a wide range of meat products. The sigmoid growth models may be more appropriate when the frequency of sampling permits the precise estimation of lag time (such as in broth tests), but in this type of study, the precise estimation of lag time is not possible. In addition, the sigmoid models were not useful in accurately calculating lag values, maximal growth rates, or maximum cell numbers when bacteriostatic conditions existed. The complex nature of the data obtained from the sigmoid functions would have made the analysis and the use of the data more difficult.

The *L. monocytogenes* growth model was subsequently validated by comparison with data generated from inoculation studies of commercial cured meat products including turkey-pork wieners, cotto salami, smoked-cooked ham, and light bologna (pork and chicken). Comparisons of the predicted *L. monocytogenes* growth rates with the actual growth rates were evaluated by using the bias factor ($10^{(\Sigma \log(\text{predicted count/observed count})/n)}$) and the accuracy factor ($10^{(\Sigma |\log(\text{predicted count/observed count})|/n)}$) of Ross *(19).* These performance factors were calculated only for control products because they could not be calculated for products exhibiting zero predicted or observed listeria growth. Bias factors indicate whether, on average, the actual values lie above or below the line of equivalence and by how much. That is, bias factors greater than 1 indicate that on average the predicted values exceed the observed values, whereas bias factors less than 1 indicate that on average the observed values exceed the predicted values. Accuracy factors evaluate how much the observed values deviate from the predicted values and are roughly equivalent to a 50% confidence interval *(19).*

RESULTS AND DISCUSSION

Product formulations. The meat blocks for the various treatments contained combinations of pork trimmings (42% lean) and ham muscles. Products formulated to contain 55% finished-product moisture contained a pork/ham ratio of about 1:1. Treatments formulated to contain 74% moisture contained a pork/ham ratio of approximately 0.17. The treatment formulated to contain 83.5% moisture required the addition of trimmed turkey breast halves to achieve the compositional target. The analytical values for salt, lactate, diacetate, and finished-product moisture were compared with the target values (Table 1) and were not found to be different; consequently, no adjustments were made to the coefficients for the analysis of the data.

Analysis of variance. The growth rate data obtained from the nonlinear regressions are presented in Table 2 along with the design coefficients for each run (treatment). Note that the growth rate constants ranged from 0 to 0.1338, and the rate constants for the center point runs were all 0. Thus, total error, rather than pure error, was used as the test term in an analysis of variance (Table 3). Consequently, the lack of fit could not be calculated. The analysis of variance indicated that the use of potassium lactate syrup significantly influenced the rate of *L. monocytogenes* growth (Table 3). Finished-product moisture and sodium diacetate content also influenced the *L. monocytogenes* growth rate, but not as much as potassium lactate did ($P =$

0.105, 0.11, and 0.0004, respectively). The regression coefficients associated with these main effects also indicated that the use of increasing amounts of sodium diacetate and potassium lactate syrup resulted in a decrease in *L. monocytogenes* growth rate constants, whereas the use of increasing amounts of finished-product moisture increased *L. monocytogenes* growth rate constants (Table 4). Although not a significant factor in this study ($P > 0.30$), sodium chloride also had a negative correlation coefficient. Shelef and Yang *(23)* noted that in their study, at the concentrations typically used in food, NaCl did not affect the growth of *L. monocytogenes.*

Interactions were not significant ($P > 0.2$), except for that of sodium diacetate and potassium lactate syrup, which was significant at $P < 0.10$. However, inclusion of this interaction in the final model did not improve the fit significantly, so it was not used.

These results are consistent with what was expected with regard to the use of these ingredients as antimicrobial agents in cured RTE meat products. Sodium diacetate has been shown to suppress the growth of *L. monocytogenes (22).* Sodium lactate has been shown to reduce the rate of *L. monocytogenes* growth, especially in combination with sodium acetate *(6).* Increased amounts of finished-product moisture increased the potential for *L. monocytogenes* growth (Table 4). Shelef and Yang *(23)* have suggested that there is a complex interaction between water activity and product moisture content and that lactate may be less effective in products with higher moisture levels.

The final regression equation predicting the growth of *L. monocytogenes* in cured RTE meat products stored at 4°C is as follows:

$$\begin{aligned} &\text{Predicted } L.\ monocytogenes \text{ growth rate} \\ &= 0.07979 - 0.00595\cdot[\text{NaCI}(\%)] \\ &\quad - 0.13692\cdot[\text{Diacetate }(\%)] \\ &\quad - 0.03490\cdot[\text{Potassium lactate syrup }(\%)] \\ &\quad + 0.00074\cdot[\text{Product moisture }(\%)] \\ &\quad + 0.00286\cdot[\text{Potassium lactate syrup }(\%)]^2 \end{aligned}$$

Validation of model. We used predictive model performance factors *(19)* and a simple linear regression analysis to evaluate the model generated in this study. Statistical validation of microbial growth models for food products is complex. In contrast to broth systems, the biological variability, the analytical variation, the inoculation level, and other complicating factors of food systems make it difficult to fully evaluate how well predictions obtained from experimental data fit actual product data. For example, Heiszler et al. *(10)* demonstrated that interactions between end point cooking temperature and smoke density could affect microbial growth on frankfurters. The products used for validation of this model differed with regard to amount and type of smoke deposited, physical size, and a host of other attributes, including meat particle size, presence of added flavorings, and product proximate composition. The model derived for this study was verified for accuracy by comparison with actual *L. monocytogenes* growth data from independent challenge tests conducted with similar storage conditions for four different commercial RTE meat products made in the Oscar Mayer Division Research Pilot Plant. Furthermore, inoculation levels used in the validation samples were 3 $\log_{10}$ CFU/g lower than those used in the original RSM experiment.

It was difficult to apply the performance factors (bias factor and accuracy factor) of Ross *(19)* to our data, since we were deliberately striving to attain low to no growth. We could not calculate the performance factors when either actual or predicted growth rates equaled zero. Others *(12)* have also encountered this difficulty. The use of generation time resulted in the same problem, since the counts at the end of the storage time were the same as the initial counts, and generation time could not be calculated. Our goal was to use data derived from a separate experiment (the RSM experiment) and apply it to cured products that differed drastically in composition. Consequently, performance factors were calculated and evaluated for control products (those containing neither lactate nor diacetate); the results are displayed in Table 5. A bias factor of 1.24 indicated that on average the predicted *L. monocytogenes* growth exceeded the observed values by about 24% *(19).* The accuracy factor, a measure of average deviation between observed and predicted values, was 1.97, indicating that the scatter of the data was about twice as extensive as that of the predicted values *(19).* The accuracy factor of data in perfect agreement would be 1. Good agreement ($r^2 = 76$; standard error of estimate = 1.08 $\log_{10}$ CFU/g) was observed when all growth data were combined and the observed values versus the predicted values were plotted (Fig. 1).

Graphs of the predicted versus the observed *L. monocytogenes* counts for all validation samples are shown in Figures 2 through 5. For all validation products, control samples (containing 0% potassium lactate and 0% sodium diacetate) allowed proliferation of the *L. monocytogenes* inoculum over the 18-week test period. Predicted *L. monocytogenes* counts for control samples of all products agree relatively closely with the observed values except that the observed *L. monocytogenes* growth exceeded the predicted growth at 2, 4, and 6 weeks by 0.45, 0.31, and 0.28 $\log_{10}$ CFU/g, respectively, for light bologna. The difference was >2.5 $\log_{10}$ CFU/g after 8 weeks and diminished to 0.64 $\log_{10}$ CFU/g after 18 weeks of storage (Fig. 2). Observed *Listeria* growth fluctuated around predicted growth for cotto salami (Fig. 3) until after 12 weeks, at which time the difference exceeded 1.77 $\log_{10}$ CFU/g. The wieners exhibited an inconsistent growth pattern over the testing period for control samples, which may be attributable to factors not incorporated into this study (e.g., smoke density [see above]; Fig. 5). However, *L. monocytogenes* levels did increase from 1.3 $\log_{10}$ CFU/g (<20 CFU/g) to 6 $\log_{10}$ CFU/g over the test period (Fig. 5).

The growth of *L. monocytogenes* was drastically reduced in each product with the addition of potassium lactate and sodium diacetate (Figs. 2 through 5). Treatment 2 (1.5% potassium lactate and 0.15% sodium diacetate) and

treatment 3 (2.5% potassium lactate and 0.15% sodium diacetate) limited the growth of *L. monocytogenes* in all products throughout the 18-week testing period in the validation study (Figs. 2 through 5). Even though there were sporadic increases in plate counts for some treatments, there were no increases in CFU per gram from the time of the inoculation of the products to the end of the study (18 weeks).

CONCLUSIONS

The goal of this project was to provide a useful model for determining the target amounts of sodium diacetate and potassium lactate for cured meat product formulations to inhibit the growth of *L. monocytogenes*. Such calculations also require knowledge of the finished-product NaCl and moisture contents to provide a model robust enough to cover a multitude of cured meat products. The model studied here has been validated and has been found to be useful for determining the proper sodium diacetate and potassium lactate amounts. However, it must be recognized that the results presented here are specific to particular products designed for this study and for the *L. monocytogenes* strains used. Testing in other environments and with *Listeria* spp. from other sources may result in different maximum growth rates. Application of this model to formulations that are outside the model's limits is inappropriate. This model (i) illustrates that *L. monocytogenes* is capable of growing in cured meat products and (ii) demonstrates that the listeria growth rate can be diminished if appropriate amounts of lactate and diacetate are added to these products.

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