

ABSTRACT

The effects of temperature, lactic acid (or pH), sodium chloride, and sodium nitrite on the non-thermal inactivation of a three strain mixture of *Listeria monocytogenes* were examined in brain heart infusion broth. A total of 249 survivor curves representing 157 combinations of the four variables were generated. The survivor curves were described mathematically by fitting data using linear and nonlinear primary models. Supplemental studies demonstrated that (1) preculcating the microorganism in an acidic environment or in media containing glucose increased acid tolerance, (2) survivor curve tailing was not due to the presence of a more resistant subpopulation, and (3) the rate of non-thermal inactivation was independent of initial population density. Response surface models were developed for predicting the effects and interactions of the four independent variables on the inactivation of *Listeria monocytogenes* under adverse environmental conditions.

Key Words: microbes, inactivation, listeria, mathematical model

INTRODUCTION

LIKE OTHER foodborne pathogens, when *Listeria monocytogenes* is placed in an adverse environment, it will be inactivated at a rate that is dependent on the severity of the conditions and the physiological characteristics of the species. In general, the environmental and cultural factors that influence the growth of bacteria in foods also influence their inactivation. This includes well recognized parameters such as storage temperature, pH, acidulant identity, water activity, humectant identity, antimicrobials, etc. With *Listeria monocytogenes*, the rate of non-thermal inactivation is dependent on most of these factors (Ahmad and Marth, 1989; El-Shenawy and Marth, 1989; Sorrells et al., 1989; Sorrells and Enigl, 1990; Cole et al., 1990; Buchanan et al., 1993).

While the kinetics of microbial inactivation in response to thermal processing has been studied extensively, there has been relatively little reported quantitative data on nonthermal inactivation. Using *Listeria monocytogenes* as an example of a relatively hardy, vegetative foodborne pathogen, the objective of our study was to examine quantitatively the effects and interactions of temperature, sodium chloride (water activity), lactic acid (or pH), and sodium nitrite on aerobic inactivation. These data were then used to (1) evaluate the effectiveness of two primary models for depicting survivor curves, and (2) assess the feasibility of developing secondary models for predicting the impact of the same variables on the rate of *L. monocytogenes* inactivation.

MATERIALS & METHODS

Microorganisms

A three strain mixture of *Listeria monocytogenes* (Scott A, HO-VJ-S, and V-7) was used. Stock cultures of each strain were maintained in Brain Heart Infusion Broth (BHI) (Difco, Detroit, MI) stored at 5°C. Starter cultures of each strain were initiated by inoculating individual 250-mL Erlenmeyer flasks containing 25 mL of BHI + 0.3%

dextrose and incubating on a rotary shaker (150 rpm) for 24 hr at 37°C. The three cultures were then combined to provide an inoculum containing about 10⁹ CFU/mL for each of the strains.

Preparation of test system

BHI was supplemented with crystalline NaCl and 85% lactic acid to achieve concentrations of 0.5–19.0% W/V and 0.0–2.0% W/V, respectively. The concentrations of NaCl or NaNO₂ influenced the measured pH of the medium; however, the effect was relatively slight (Table 1). The media were dispensed in 20 mL portions to dilution bottles, and the pH recorded. The bottles were closed with a screw-cap and sterilized by autoclaving. Immediately prior to inoculation, filter-sterilized NaNO₂ was added to achieve levels of 0–200 µg/mL. The volume (up to 0.8 mL) of the nitrite solution and the inoculum (see below) were taken into account in the initial preparation of the medium.

Inactivation studies

Each bottle was inoculated with 0.6 mL of the combined 24 hr culture to achieve an initial population density of ≈ 10⁸ CFU/mL. The caps were loosened, and the bottles incubated horizontally to maximize oxygen transfer. The bottles were stored without agitation at 4, 12, 19, 28, 37, or 42°C. Periodically, 0.1 mL samples were removed aseptically, diluted as needed in 0.1% peptone water, and surface plated on duplicate Tryptose agar (Difco) plates using either a Spiral Plater (Spiral Systems, Inc., Cincinnati, OH) or spread plates, depending on the level of surviving cell anticipated. All plates were incubated for 24 hr at 37°C, and enumerated using an automated colony counter (Model 500A, Spiral Systems, Inc.). Sampling was continued for 90 days or until counts fell below the lower limit of detection (log # < 1.03 CFU/mL).

Survivor curves

Survivor curves were generated by fitting the data to two primary models. In both cases, curve fitting was performed using ABACUS, a nonlinear regression program that employs a Gauss-Newton iterative procedure (Buchanan and Phillips, 1990). The first model (Eq. 1) is a simple linear relationship that includes a term to take into account the existence of a lag period prior to the initiation of an exponential decline in population (Buchanan et al., 1993).

$$Y = Y_0 \quad [t < t_L] \quad (1)$$

$$Y = Y_0 + s(t - t_L) \quad [t \geq t_L]$$

where: Y = Log₁₀ count of bacteria at time t, Log(CFU/mL); Y₀ = Log₁₀ count of bacteria at time t = 0, Log(CFU/mL); s = Slope of the survivor curve, [Log(CFU/mL)]/hr; t = Time, hr; t_L = Duration of lag period prior to initiation of inactivation, hr.

D-values were calculated by taking the negative reciprocal of s. The "time to a 4-D (99.99%) inactivation" (t_{4-D}) values were calculated using the equation,

$$t_{4-D} = t_L + (4 \cdot D) \quad (2)$$

The second primary model is a logistics-based equation. It was developed to describe the kinetics when there was significant tailing, possibly due to existence of a more resistant subpopulation (Whiting and Buchanan, 1992).

$$\text{Log}[Y/Y_0] = \text{Log}_{10} \left[\frac{F_1(1 + e^{-b_1 t})}{1 + e^{b_1(t-t_L)}} + \frac{(1 - F_1)(1 + e^{-b_2 t})}{(1 + e^{b_2(t-t_L)})} \right] \quad (3)$$

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Table 1—Effect of temperature, sodium chloride, lactic acid, and sodium nitrite on the kinetics nonthermal inactivation of *Listeria monocytogenes* based on survivor curves generated using linear and nonlinear primary models

Independent variables					LINEAR MODEL					NON-LINEAR MODEL				
T	S	L	N	n	pH	Y ₀	t _l	D	t _{4-D}	t _l	F ₁	D ₁	D ₂	t _{4-D}
42	0.5	0.50	0	2	4.1	8.5	6	7.6	36.5	6	-4.0	5.3	35.0	27.4
42	0.5	0.50	100	1	3.8	7.2	0	0.9	3.6	0	-7.0	0.9	0.0	3.8
42	0.5	1.00	50	1	3.5	7.3	0	0.9	3.5	0	-7.0	0.9	0.0	3.7
42	0.5	2.00	0	1	3.1	8.5	0	0.5	2.1	0	-7.0	0.5	0.0	2.1
42	6.3	1.50	150	1	3.3	8.1	0	0.5	2.0	0	-7.0	0.5	0.0	2.0
42	6.3	2.00	0	1	3.1	8.2	0	0.5	2.0	0	-7.0	0.5	0.0	2.0
42	10.3	0.00	10	3	7.1	8.7	0	62.5	250.0	0	-7.0	59.6	1.0	256.8
42	10.3	0.00	200	1	7.1	8.8	52	37.5	202.2	50	-7.0	37.4	0.0	200.7
42	10.3	0.25	0	1	6.1	7.2	0	61.7	246.7	0	-7.0	59.6	0.0	256.6
42	10.3	0.50	20	2	4.1	8.6	0	8.5	34.1	6	-4.0	4.9	45.0	25.9
42	10.3	0.50	100	1	4.5	7.9	0	1.0	4.1	0	-7.0	1.0	0.0	4.2
42	10.3	1.00	10	3	3.5	8.3	0	0.4	1.8	0	-7.0	0.4	0.0	1.8
42	10.3	1.00	200	1	3.9	8.0	0	0.8	3.0	0	-7.0	0.7	0.0	3.1
42	10.3	1.50	5	2	3.4	8.2	0	0.4	1.7	0	-7.0	0.7	0.0	2.8
42	13.1	0.50	0	1	3.8	5.0	2	11.0	46.1	2	-7.0	10.4	0.0	45.7
42	13.1	1.00	100	1	3.5	7.9	0	0.5	2.0	0	-7.0	0.5	0.0	2.1
42	19.0	0.25	0	1	6.1	7.1	0	14.1	56.3	0	-7.0	13.5	0.0	58.3
42	19.0	0.50	50	1	3.8	7.9	0	0.8	3.2	0	-7.0	0.8	0.0	3.3
42	19.0	0.25	200	1	6.1	6.0	0	16.4	65.6	0	-7.0	15.7	0.0	67.4
42	19.0	1.50	50	1	3.3	7.9	0	0.5	2.1	0	-7.0	0.5	0.0	2.1
37	0.5	0.50	200	1	3.9	8.2	0	0.9	3.5	0	-7.0	0.8	0.0	3.6
37	0.5	1.00	100	1	3.5	8.4	0	0.8	3.0	0	-7.0	0.7	0.0	3.1
37	0.5	2.00	0	2	3.2	8.2	0	1.2	4.9	0	-7.0	1.1	0.0	4.9
37	6.3	1.00	200	1	3.9	7.8	0	0.8	3.2	0	-7.0	0.8	0.0	3.2
37	6.3	2.00	0	2	3.2	8.2	0	1.2	4.9	0	-7.0	1.1	0.0	4.9
37	10.3	0.00	10	3	7.4	8.2	0	63.9	255.6	0	-7.0	61.6	0.0	265.2
37	10.3	0.25	200	1	4.5	8.2	0	9.4	37.7	0	-7.0	9.0	0.0	38.8
37	10.3	0.50	0	1	3.9	8.1	0	6.1	24.4	0	-7.0	5.8	0.0	25.1
37	10.3	0.50	50	1	3.9	8.1	0	6.0	23.8	0	-7.0	5.7	0.0	24.6
37	10.3	0.50	50	1	3.9	8.1	0	6.0	23.8	0	-7.0	5.7	0.0	24.6
37	10.3	1.00	10	3	3.9	7.7	0	1.4	5.6	0	-4.0	0.8	20.0	3.4
37	10.3	1.00	150	1	3.3	7.9	0	0.7	2.8	0	-7.0	0.7	0.0	2.9
37	10.3	1.00	200	1	3.9	7.9	0	0.8	3.1	0	-7.0	0.7	0.0	3.1
37	13.1	0.50	0	1	4.2	6.9	0	24.4	97.6	0	-7.0	23.5	0.0	101.1
37	19.0	0.00	200	1	7.3	8.7	66	38.2	152.8	0	-7.0	36.2	0.0	155.6
37	19.0	0.50	200	1	4.2	8.0	0	0.8	3.1	0	-7.0	0.7	0.0	3.2
28	0.5	0.50	0	1	4.2	7.1	153	89.0	508.9	152	-7.0	88.2	0.0	505.5
28	0.5	0.50	200	1	4.2	7.1	0	23.8	95.2	0	-7.0	22.8	0.0	98.1
28	4.5	0.40	50	1	5.0	7.9	0	249.6	998.3	0	-4.0	148.8	909.2	640.9
28	4.5	0.50	200	1	3.8	6.3	0	1.3	5.2	0	-4.2	1.2	17.4	4.6
28	4.5	1.00	150	1	3.3	8.1	0	0.8	3.4	0	-7.0	0.8	0.0	3.4
28	6.3	0.00	5	3	7.3	8.2	>2500	NI*	—	>2500	—	NI	—	—
28	6.3	0.00	15	3	7.3	8.0	>2500	NI	—	>2500	—	NI	—	—
28	6.3	0.00	200	1	7.3	8.7	>2000	NI	—	>2500	—	NI	—	—
28	6.3	0.37	5	3	4.0	8.4	20	78.1	332.4	30	-7.0	75.2	0.0	341.9
28	6.3	0.37	15	3	4.0	8.6	0	59.5	227.6	0	-6.9	59.5	0.0	256.0
28	6.3	0.75	0	1	3.6	6.7	0	27.2	108.6	0	-7.0	25.6	0.0	110.2
28	6.3	0.75	50	1	3.6	8.3	0	1.3	5.2	0	-3.7	0.8	24.4	3.3
28	6.3	0.75	75	1	3.6	8.1	0	1.1	4.4	0	-4.4	0.6	24.2	2.4
28	6.3	1.50	5	1	3.2	8.1	0	40.0	160.0	0	-7.0	19.1	0.0	82.3
28	6.3	1.50	15	1	3.2	7.9	0	15.9	63.5	0	-7.0	14.3	0.0	61.5
28	7.8	0.40	50	1	5.0	7.7	0	66.7	266.7	0	-7.0	61.4	0.0	264.2
28	10.3	0.50	15	2	4.2	8.1	0	42.6	170.3	0	-7.0	39.8	0.0	171.4
28	10.3	0.75	100	1	3.6	8.1	0	5.7	22.8	0	-7.0	6	0.0	23.9
28	10.3	1.00	0	1	3.9	6.9	0	14.7	58.8	0	-7.0	14	0.0	60.6
28	10.3	1.00	200	1	3.9	5.9	0	4.1	16.3	0	-7.0	4	0.0	17.1
28	13.1	0.00	5	3	7.3	8.0	127	53.4	340.8	127	-7.0	53.7	0.0	341.7
28	13.1	0.00	15	3	7.3	8.0	21	48.2	213.7	11	-7.0	48.3	0.0	213.9
28	13.1	0.00	150	2	7.3	8.6	26	124.8	525.3	18	-7.0	121.3	0.0	532.4
28	13.1	0.37	5	3	4.4	7.9	175	46.7	361.7	165	-7.0	48.1	0.0	357.6
28	13.1	0.37	15	3	4.0	8.5	0	55.0	220.1	0	-7.0	50.1	0.0	215.3
28	13.1	0.75	0	1	3.6	6.3	0	28.3	113.3	0	-7.0	26.5	0.0	114.3
28	13.1	0.75	75	1	3.6	8.1	0	2.9	11.5	0	-7.0	2.8	0.0	12.0
28	13.1	1.00	50	1	3.5	8.0	0	2.9	11.7	0	-7.0	2.8	0.0	12.1
28	13.1	1.00	150	1	3.5	8.0	0	0.7	3.0	0	-7.0	0.7	0.0	3.0
28	13.1	1.50	5	1	3.3	7.3	0	17.9	71.4	0	-7.0	16.7	0.0	71.9
28	13.1	1.50	15	3	3.3	7.9	0	1.0	3.9	0	-7.0	0.9	0.0	3.8
28	13.1	1.50	50	1	3.7	6.9	0	1.0	4.1	0	-7.0	1.0	0.0	4.2
28	13.1	2.00	0	2	3.3	7.9	0	0.9	3.5	0	-3.5	0.6	4.5	2.6
28	13.1	2.00	20	2	3.3	6.9	0	1.5	5.8	0	-3.5	0.7	5.3	2.9
28	19.0	0.00	0	1	7.3	8.5	167	47.5	356.5	170	-7.0	47.0	0.0	357.9
28	19.0	0.00	200	1	7.3	8.6	117	44.4	294.3	104	-7.0	45.4	0.0	286.2
28	19.0	1.50	0	2	3.5	7.4	0	1.4	5.5	0	-7.0	1.3	0.0	5.6
19	0.5	1.00	5	2	3.8	8.1	0	40.0	160.0	4	-7.0	38.1	0.0	166.1
19	0.5	1.00	10	3	4.0	7.9	0	66.9	267.5	0	-7.0	62.5	0.0	269.2
19	0.5	1.00	50	1	3.6	8.1	0	14.1	56.3	0	-4.4	6.4	92.7	27.3
19	0.5	2.00	200	1	3.9	7.9	0	1.0	4.1	0	-7.0	1.0	0.0	4.1
19	0.5	2.00	0	1	3.4	7.0	0	14.7	58.8	0	-7.0	14.1	0.0	60.7
19	0.5	2.00	50	1	3.2	8.1	0	0.9	3.6	0	-5.1	0.4	18.3	1.7
19	4.5	0.40	50	1	5.0	7.5	146	200.3	947.2	178	-7.0	190.5	0.0	950.0
19	4.5	1.00	100	1	3.6	5.4	0	52.0	208.0	0	-4.1	49.5	87.6	213.3
19	6.3	0.50	20	2	4.0	7.9	0	51.3	205.3	0	-7.0	48.5	0.0	208.7
19	6.3	1.00	100	2	3.9	8.1	0	1.6	6.4	0	-4.6	1.4	34.1	6.2

Table 1—Continued

Independent variables							LINEAR MODEL				NON-LINEAR MODEL				
T	S	L	N	n	pH	Y ₀	t _l	D	t _{4-D}	t _l	F ₁	D ₁	D ₂	t _{4-D}	
19	6.3	1.50	0	2	3.5	8.0	0	19.0	76.2	0	-7.0	15.8	0.0	68.1	
19	7.8	0.40	50	1	5.0	7.8	0	113.7	454.8	0	-7.0	105.9	0.0	455.9	
19	10.3	1.00	10	3	3.5	7.7	0	55.6	222.2	0	-7.0	50.3	0.0	216.7	
19	10.3	1.00	0	3	3.6	7.7	0	58.8	235.3	0	-7.0	54.5	0.0	234.5	
19	10.3	1.00	20	3	3.4	7.1	0	11.7	46.7	0	-7.0	11.0	0.0	47.6	
19	10.3	2.00	10	5	3.2	7.7	0	2.0	8.0	0	-5.7	1.7	11.1	7.1	
19	13.2	0.00	15	3	7.3	8.1	0	333.3	1333.3	0	-7.0	302.5	0.0	1302.4	
19	13.1	0.25	200	1	4.6	8.2	0	63.0	252.1	0	-7.0	59.8	0.0	257.5	
19	19.0	0.00	0	1	7.3	8.5	126	250.6	1128.0	139	-7.0	244.0	0.0	1141.6	
19	19.0	0.37	10	2	4.2	7.8	0	43.5	173.9	0	-7.0	41.0	0.0	176.4	
19	19.0	0.50	10	2	4.0	8.0	0	40.8	163.3	0	-7.0	39.1	0.0	168.3	
19	19.0	1.00	0	1	3.9	6.0	0	27.0	108.1	0	-7.0	26.0	0.0	111.9	
19	19.0	1.00	10	3	3.4	7.9	0	2.6	10.5	0	-6.9	2.4	0.0	10.4	
19	19.0	1.00	100	2	4.0	8.4	0	0.9	3.6	0	-6.5	0.8	0.0	3.5	
19	19.0	1.00	200	1	3.9	7.3	0	1.4	5.5	0	-4.5	0.5	26.4	2.3	
19	19.0	2.00	0	1	3.2	8.1	0	1.0	4.0	0	-4.7	0.5	26.2	2.2	
12	0.5	0.25	0	1	6.1	7.9	0	1006.3	4025.4	0	-7.0	837.3	0.0	3605.3	
12	0.5	0.25	200	1	6.1	7.9	>2000	NI ^b	—	>2000	—	NI	—	—	
12	0.5	2.00	0	2	3.3	7.5	0	32.8	131.2	0	-7.0	31.0	0.0	133.7	
12	0.5	2.00	15	2	3.3	8.1	0	2.4	9.6	2	-2.5	1.2	25.0	6.9	
12	0.5	0.40	50	1	5.0	7.8	0	666.7	2666.7	0	-7.0	654.7	0.0	2819.2	
12	4.5	0.75	150	1	3.7	7.1	0	1.7	6.6	0	-7.0	1.5	0.0	6.4	
12	6.3	0.50	5	3	4.3	8.0	195	155.8	818.5	246	-7.0	142.4	0.0	817.5	
12	6.3	0.50	15	3	4.3	7.9	0	166.7	666.7	0	-7.0	166.0	0.0	714.9	
12	6.3	0.50	200	1	4.5	6.9	0	90.9	363.6	0	-7.0	78.7	0.0	338.8	
12	6.3	1.00	50	1	3.6	7.7	0	35.4	141.6	0	-3.0	34.0	54.6	146.4	
12	6.3	1.00	200	1	3.9	6.9	0	14.7	58.8	0	-7.0	14.3	0.0	61.4	
12	6.3	1.50	5	3	3.4	7.7	0	21.9	87.7	0	-7.0	19.4	0.0	83.6	
12	6.3	1.50	15	3	3.4	7.5	0	57.7	230.9	0	-7.0	54.1	0.0	233.1	
12	6.3	2.00	200	1	3.4	7.2	0	1.3	5.3	0	-4.0	0.6	10.4	2.7	
12	7.8	0.40	50	1	5.0	7.8	0	222.6	890.5	0	-7.0	206.8	0.0	890.5	
12	13.1	0.50	5	3	4.2	7.6	0	125.0	500.0	0	-7.0	111.1	0.0	478.4	
12	13.1	0.50	15	3	4.0	7.8	0	152.0	607.8	0	-3.0	139.6	569.0	600.2	
12	13.1	0.50	75	1	3.9	7.6	0	27.9	111.5	0	-2.8	26.5	52.3	114.1	
12	13.1	0.50	200	1	4.5	8.0	0	1.1	4.2	0	-7.0	1.0	0.0	4.3	
12	13.1	1.00	50	1	3.6	7.7	0	25.4	101.6	0	-3.5	24.2	57.5	104.1	
12	13.1	1.00	200	1	3.9	6.7	0	1.2	4.7	0	-7.0	0.9	0.0	4.0	
12	13.1	1.50	5	3	3.5	7.0	0	41.7	166.7	0	-6.9	38.9	0.0	167.4	
12	23.1	1.50	15	3	3.4	7.8	0	21.0	83.9	0	-7.0	19.7	0.0	84.9	
12	13.1	2.00	50	1	3.2	7.2	0	1.2	5.0	0	-7.0	1.2	0.0	5.0	
12	13.1	2.00	200	1	3.4	6.7	0	1.2	4.7	0	-7.0	0.9	0.0	3.8	
12	19.0	0.00	15	3	7.4	8.2	0	388.9	1555.6	0	-7.0	380.4	0.0	1638.0	
4	0.5	0.00	200	1	7.3	8.3	>2200	NI	—	>2200	—	NI	—	—	
4	0.5	0.37	0	2	4.3	8.1	462	80.7	784.9	471	-7.0	80.0	0.0	791.8	
4	0.5	0.50	0	2	4.2	8.1	421	98.3	814.8	428	-7.0	98.1	0.0	821.1	
4	0.5	0.50	100	1	3.8	5.4	24	75.8	327.2	24	-7.0	72.1	0.0	324.5	
4	0.5	0.75	150	1	3.7	7.7	0	1.7	6.6	0	-3.8	1.2	14.0	5.1	
4	0.5	0.50	200	1	4.5	8.1	0	11.6	46.5	0	-7.0	11.1	0.0	47.9	
4	0.5	1.50	200	1	3.7	7.8	0	1.1	4.4	0	-7.0	1.0	0.0	4.5	
4	0.5	2.00	0	1	3.4	7.9	0	12.0	48.2	0	-7.0	12.0	0.0	51.5	
4	0.5	2.00	50	1	3.2	7.6	0	1.1	4.2	0	-5.0	0.5	25.8	2.1	
4	0.5	2.00	100	1	3.4	7.7	0	1.0	3.8	0	-7.0	0.9	0.0	3.9	
4	4.5	0.40	50	1	5.0	7.9	0	735.1	2940.5	0	-7.0	734.3	0.0	3161.9	
4	6.3	0.50	200	1	4.5	7.1	0	11.5	46.0	0	-7.0	12.1	0.0	52.1	
4	6.3	1.00	200	1	3.6	7.8	0	1.3	5.1	0	-4.9	1.0	21.0	4.1	
4	6.3	2.00	0	1	3.4	7.1	0	10.5	42.1	0	-7.0	11.0	0.0	47.2	
4	6.3	2.00	100	1	3.2	7.6	0	0.9	3.6	0	-5.9	0.4	30.8	1.6	
4	7.8	0.40	50	1	5.0	7.9	0	386.8	1547.3	0	-2.5	221.3	914.0	953.0	
4	10.3	0.00	10	3	7.1	8.2	>3900	NI	—	>3900	—	NI	—	—	
4	10.3	0.00	150	1	7.3	9.0	>2200	NI	—	>2200	—	NI	—	—	
4	10.3	0.50	100	1	4.0	7.7	0	95.3	381.3	0	-7.0	89.5	0.0	385.3	
4	10.3	0.50	200	1	4.5	7.0	0	10.5	42.1	0	-7.0	10.9	0.0	46.8	
4	10.3	1.00	10	3	3.5	7.8	0	28.1	112.4	0	-2.9	26.0	186.7	111.8	
4	10.3	1.50	50	1	3.4	7.8	0	1.3	5.4	0	-4.4	1.0	17.4	4.1	
4	13.1	1.00	0	2	3.8	8.1	0	71.4	285.7	0	-7.0	68.4	0.0	294.3	
4	13.1	1.00	100	1	3.6	7.8	0	2.4	9.6	0	-4.0	2.1	23.2	9.1	
4	13.1	1.00	200	1	3.6	7.8	0	1.2	4.8	0	-4.7	0.8	20.4	3.3	
4	13.1	1.50	0	1	3.4	6.7	4	50.7	206.6	4	-7.0	48.0	0.0	208.9	
4	19.0	0.00	0	2	7.3	8.1	>4000	NI	—	>4000	—	NI	—	—	
4	19.0	0.50	200	1	4.5	7.8	0	1.3	5.2	0	-4.4	1.0	25.0	4.1	
4	19.0	1.50	0	2	3.5	7.8	0	16.1	64.5	0	-6.9	15.2	0.0	65.4	
4	19.0	1.50	100	1	3.4	7.5	0	1.0	3.9	0	-5.3	0.4	21.2	1.9	
4	19.0	1.50	200	1	3.5	7.7	0	0.9	3.5	0	-7.0	0.8	0.0	3.6	
4	19.0	2.00	0	2	3.4	7.6	0	16.4	65.6	0	-6.9	15.4	0.0	66.3	
4	19.0	2.00	20	2	3.4	7.6	0	17.2	69.0	0	-6.9	16.1	0.0	69.4	
4	19.0	2.00	200	1	3.4	8.0	0	1.1	4.3	0	-7.0	1.0	0.0	4.3	

* T = temperature (°C), S = sodium chloride (%), L = lactic acid (%), N = sodium nitrite (µg/mL), n = number of replicates, t_l = lag period prior to inactivation (hr), D = D-value (90% decline) (hr), t_{4-D} = time to 4-D (99.99%) inactivation (hr), F₁ = fraction of initial population in major group, Y₀ = initial population density (Log[cfu/mL]).
^b NI = Less than 1 log cycle of inactivation over the course of the experimental period.

where: $b_1 = 2.3/D_1$ = inactivation rate for major group; $b_2 = 2.3/D_2$ = inactivation rate for minor group; F_1 = fraction of initial population in major group; $(1-F_1) = F_2$ = fraction of population in minor subgroup.

D-values were calculated using the relationships depicted above. t_{4-D} -values were calculated based on the D_1 and t_L values only, using the following equation.

$$t_{4-D} = \frac{\text{LN} [(1 + e^{-b_1 t_L})/0.0001] - 1}{b_1} + b_1 \cdot t_L \quad (4)$$

Assessment of tailing

An assessment of the genetic basis of the apparent increased resistance associated with tailing of inactivation curves was evaluated using a variable combination of 6.3% NaCl, 1.0% lactic acid, 100 $\mu\text{g/mL}$ NaNO_2 , and 19°C. These conditions gave a substantial, reproducible tailing. The initial culture was inoculated, held for 120 hr, and sampled periodically as described previously. An isolated colony from one of the final enumeration plates was recultured in BHI for 24 hr at 37°C. The inactivation kinetics of the isolate were then determined under conditions (6.3% NaCl, 1.0% lactic acid, 100 $\mu\text{g/mL}$ NaNO_2 , and 19°C) identical to the initial inactivation.

Effect of pH history of the starter culture

The effect of the pH of the inocula on the subsequent resistance of *L. monocytogenes* to acid conditions was assessed using three media; BHI, BHI + 0.3% dextrose, and Tryptic Soy Broth without glucose (TSB-G) (Difco). The media were adjusted to various pH values using concentrated HCl and then dispensed, sterilized, inoculated, and incubated for 24 hr as described. Resistance was then assessed using two variable combinations, 19°C/0.5% NaCl/1.0% lactic acid/0 $\mu\text{g/mL}$ NaNO_2 and 28°C/10.3% NaCl/1.0% lactic acid/50 $\mu\text{g/mL}$ NaNO_2 . The effect of anaerobiosis on the resistance of the starter cultures was determined by incubating the cultures in nitrogen-flushed, sealed trypticizing flasks (Buchanan et al., 1989b).

Effect of inoculum size

The effect of inoculum size on inactivation kinetics of *L. monocytogenes* was examined using three variable combinations: 19°C/19.0% NaCl/1.0% lactic acid/100 $\mu\text{g/mL}$ NaNO_2 , 19°C/10.3% NaCl/1.0% lactic acid/0 $\mu\text{g/mL}$ NaNO_2 , and 28°C/13.1% NaCl/0.0% lactic acid/150 $\mu\text{g/mL}$ NaNO_2 . These variable combinations were chosen as examples of rapid, intermediate, and slow inactivation rates, respectively. Inocula levels ranged from 10^4 to 10^9 CFU/mL. The cultures were incubated and sampled.

Secondary models

Response surface analysis was used to generate secondary models on the independent variables (SAS Institute, Inc., 1989). An initial assessment of various potential transformations indicated that a natural logarithm transformation helped stabilize the variance and provided the best fit of the data. Four-variable models on temperature/NaCl/lactic acid/ NaNO_2 and temperature/NaCl/pH/ NaNO_2 were generated using both quadratic and cubic polynomials. Separate models were generated for the data sets based on linear and nonlinear primary models.

RESULTS

A TOTAL of 249 survivor curves were generated, representing 157 unique combinations of the four independent variables (Table 1). Inactivation was exponential, with the rate dependent on severity of conditions. Representative examples of the types of survivor curves are shown (Fig. 1). Some of the cultures, particularly those under less severe conditions, had lag periods prior to initiation of inactivation. Likewise, some of the variable combinations had survivor curves with distinct, reproducible tails. No clear-cut relationship between the four variables and the incidence and extent of tailing was readily apparent. Some of the variable combinations supported growth of *L. monocytogenes*, as evidenced by a 2- to 10-fold increase

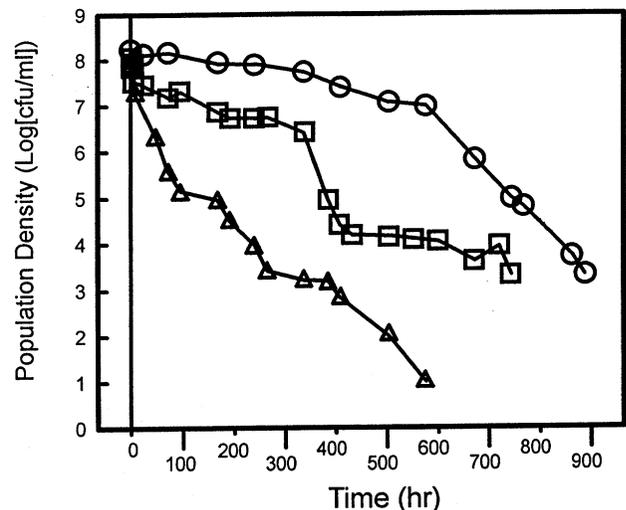


Fig. 1—Representative survivor curves for a three strain mixture of *Listeria monocytogenes*: (□) 12°C–13.1% NaCl–0.5% lactic acid–15 $\mu\text{g/mL}$ NaNO_2 (exponential with lag and tail); (○) 28°C–13.1% NaCl–0.0% lactic acid–5 $\mu\text{g/mL}$ NaNO_2 (exponential with lag); (Δ) 12°C–6.3% NaCl–0.5% lactic acid–200 $\mu\text{g/mL}$ NaNO_2 (exponential only).

in population density prior to inactivation. In those cases, the Y_0 value was fixed and the period of growth was included as part of the t_L value.

The differences in types of inactivation curves prompted trials of both linear and non-linear primary models to quantify survivor curves. Likewise, the lag periods in a few of the cultures prompted determinations of t_{4-D} values. The selection of a 4-D inactivation was based on the assumption that generally the levels of *L. monocytogenes* in a product would be < 100 CFU/g (Buchanan et al., 1989a). A 99.99% inactivation would reduce that level of *L. monocytogenes* to < 1 CFU/100 g.

Qualitatively, the more adverse the environment, the more rapid the rate of inactivation (Table 1). Storage temperature and lactic acid concentration most strongly affected survival, with *L. monocytogenes* most rapidly inactivated at high temperatures and elevated acid. High NaCl levels accelerated inactivation to a lesser degree, with some indication that lower concentrations of NaCl were protective. The effect of sodium nitrite was influenced by the other variables, particularly lactic acid content. At high lactic acid levels (low pH), sodium nitrite enhanced inactivation, whereas the nitrite had little effect when acid concentrations were low. This is consistent with sodium nitrite's pH-dependent bacteriostatic and bactericidal activity (Buchanan et al., 1989b).

Supplemental studies were conducted to determine the effect of three additional factors, inoculum size, tailing, and pH history, on the kinetics of *L. monocytogenes* inactivation. The effect of inoculum size on the kinetics of inactivation was examined for three combinations in conjunction with inocula ranging from 10^4 – 10^9 CFU/mL. Results for one set of conditions are shown (Fig. 2). Regression analysis indicated that in all three cases, the rate of inactivation over the inocula range was not dependent on inoculum level. Accordingly, inoculum level was not considered during subsequent development of secondary models.

As indicated above, tailing of survivor curves was observed with many of the variable combinations. There are several potential explanations for tailing in thermal and non-thermal inactivation, including the existence of genetically more resistant subpopulations. This possibility was evaluated by following the inactivation of a 19°C/6.3% NaCl/1.0% lactic acid/100 $\mu\text{g/mL}$ NaNO_2 culture; a condition that gave substantial, reproducible tailing (Fig. 3). A single colony from the "tail"

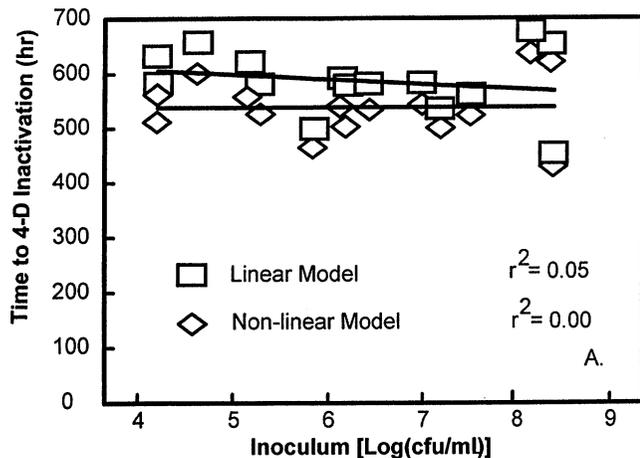


Fig. 2—Effect of initial population density on the time to achieve a 4-D inactivation of *Listeria monocytogenes* held under conditions of 28°C–13.1% NaCl–0.0% lactic acid–150 µg/mL NaNO₂.

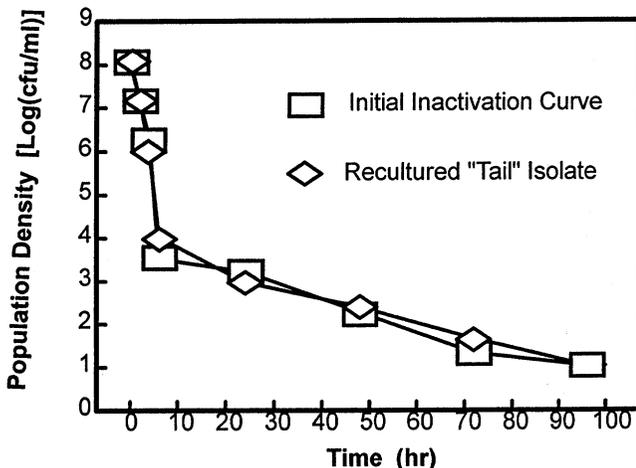


Fig. 3—Effect of reculturing a *Listeria monocytogenes* isolate from the "tail" of a 19°C–6.3% NaCl–1.0% lactic acid–100 µg/mL NaNO₂ inactivation curve on the microorganism's survival characteristics.

was recultured and its inactivation kinetics re-assessed. The kinetics of the recultured isolate were identical to the original culture, indicating that tailing had not led to selection of a more resistant subpopulation.

Reports have indicated that growing bacteria in a somewhat acidic environment enhances their ability to resist subsequent exposure to adverse acidic conditions (Foster, 1991, 1992; Foster and Hall, 1990, 1991; Kroll and Patchett, 1992). If not taken into account when generating data for a kinetic model, this would result in predictions that would considerably overestimate the rate of inactivation. The importance of cultural conditions under which the inocula were grown was assessed by growing the organism at various initial pH values using three media; BHI + 0.3% additional glucose (the medium used throughout the rest of the study for culturing the inocula), BHI without supplemental glucose (medium has 0.2% as part of normal formulation), and TSB-G. The cells were then exposed to two combinations of inactivation conditions: 19°C/0.5% NaCl/1.0% lactic acid/0 µg/mL NaNO₂ (data not shown) and 28°C/10.0% NaCl/1.0% lactic acid/50 µg/mL NaNO₂ (Table 2). No consistent difference in acid tolerance of cells grown in BHI or BHI + 0.3% glucose was observed, nor were any consistent differences associated with culturing the inocula anaerobically (data not shown). However, a large difference in general acid resistance was observed when the microorganisms

Table 2—Effect of medium composition and pH under which inocula were grown on the ability of *Listeria monocytogenes* to subsequently survive conditions of 19°C–10.0% NaCl–1.0% lactic acid–50 µg/mL NaNO₂

Medium	Atm	Initial pH	Final pH	t _L (hr)	D-value (hr)	t _{4-D} (hr)
BHI	Aerobic	7.3	6.0	0.0	23.6	94.5
		6.5	5.2	0.0	22.2	88.8
		6.0	5.0	0.0	21.2	84.8
		5.5	4.9	0.0	13.7	54.9
		5.0	4.8	0.0	18.8	75.4
BHI + 0.3% glucose	Aerobic	7.3	5.0	0.0	24.0	96.2
		6.5	5.2	0.0	25.2	100.6
		6.0	5.0	0.0	20.1	80.3
		5.5	4.9	0.0	13.3	53.0
		5.0	4.8	0.0	18.8	75.2
TSB-G	Aerobic	7.3	7.1	0.0	0.6	2.2
		6.5	5.2	0.0	7.3	29.1
		6.0	5.0	0.0	7.3	29.4
		5.5	4.9	0.0	6.8	27.0
		5.0	4.8	0.0	7.4	29.7

were initially cultured in TSB-G. Further, > 10-fold differential in resistance occurred between the pH 7.3 cultures and those that had been adjusted to lower pH levels. The different responses observed with the media appeared to be due, in part, to the presence of glucose in the BHI which resulted in a depression of pH to ≈5. This decline in pH enhanced acid resistance in a manner consistent with that observed with acidified TSB-G. These results indicated that the techniques used to initially culture the mixture of *L. monocytogenes* strains provided cells in an acid resistant form. This ensured that any model generated would be conservative in relation to acid tolerance.

Response surface models were initially generated for D, t_L, and t_{4-D}. However, during the first iteration of data generation/model development, we concluded that response surface techniques were not effective for modeling t_L due to the high percentage of variable combinations that had no lag period. While D-values could be modeled, without the corresponding t_L the inactivation rates for some conditions would be considerably overestimated. Accordingly, we decided to concentrate on development of models for t_{4-D}, with separate models generated using % lactic acid and pH as alternate variables.

The t_{4-D} data were modeled directly, as well as using square root and natural logarithm (LN) transformations. Comparisons of R² values and observed vs predicted values indicated that models based on the untransformed data or square root transformed data consistently were less effective than those based on LN transformed data. The LN-transformed t_{4-D} values generated with the linear and nonlinear primary models were fitted using quadratic (not shown) and cubic models (Table 3). The latter consistently provided better fits. Comparison of R² values (Table 3) indicated that the models based on the nonlinear data set provided a somewhat better fit than those based on the linear data set. Likewise, the models based on % lactic acid as a variable had somewhat higher R² values than the corresponding models where pH was the independent variable. Comparisons of observed vs predicted values (Fig. 4A–D) indicated that all four of the cubic models provided similar fits, though the variability appeared somewhat less with models based on the linear data set. A relatively high degree of variability was evident (Fig. 4); however, this was expected since the experimental data included inactivation times that ranged from hours to months.

In an attempt to simplify the four cubic response surface models, the data were also analyzed using backward regression with a probability >0.01 as criterion for elimination (Table 4). While the four generated models greatly simplified the polynomial expressions, comparisons of both R² values (Table 4) and agreement between observed and predicted values (not shown) indicated a substantially poorer fit.

Table 3—Cubic response surface models for the time for a 4-D inactivation of *Listeria monocytogenes**

Models based on using lactic acid as a variable:

Linear data set:

$$\text{LN}(t_{4-D}) = 7.2946 + 0.1566*T + 0.237*S - 3.2522*L + 0.00749*N - 0.00976*T*S - 0.0213*T*L - 0.000397*T*N - 0.1061*S*L - 0.000595*S*N - 0.0509*L*N - 0.00671*T^2 - 0.0178*S^2 + 1.9463*L^2 - 0.0000198*N^2 - 0.00257*T*S*L - 0.00000105*T*S*N + 0.000185*T*L*N - 0.0000228*S*L*N + 0.0000442*T*S^2 + 0.00000145*T*N^2 + 0.0108*T*L^2 + 0.000222*T^2*S + 0.00000134*T^2*N + 0.000209*T^2*L + 0.0459*S*L^2 + 0.00000224*S*N^2 + 0.00264*S^2*L - 0.000000448*S^2*N + 0.0000669*L*N^2 + 0.0141*L^2*N + 0.0000344*T^3 + 0.000364*S^3 - 0.7234*L^3 - 0.000000026*N^3 + 0.0000318*T*S*L*N$$

$$R^2 = 0.875$$

Nonlinear data set:

$$\text{LN}(t_{4-D}) = 8.4602 + 0.2183*T + 0.0519*S - 5.849*L + 0.00489*N - 0.00729*T*S - 0.0463*T*L - 0.000795*T*N + 0.0329*S*L - 0.00033*S*N - 0.0632*L*N - 0.01*T^2 - 0.00672*S^2 + 3.6469*L^2 + 0.0000258*N^2 - 0.00362*T*S*L + 0.0000101*T*S*N + 0.000347*T*L*N + 0.000158*S*L*N + 0.000151*T*S^2 + 0.00000118*T*N^2 - 0.00238*T*L^2 + 0.000168*T^2*S + 0.00000922*T^2*N + 0.00155*T^2*L + 0.00582*S*L^2 - 0.00000046*S*N^2 + 0.00222*S^2*L + 0.000000448*S^2*N + 0.0000113*L*N^2 + 0.0146*L*N + 0.0000706*T^3 - 0.0000751*S^3 - 0.9833*L^3 - 0.000000095*N^3 + 0.0000199*T*S*L*N$$

$$R^2 = 0.892$$

Models based on using pH as a variable:

Linear data set:

$$\text{LN}(t_{4-D}) = -38.4809 + 0.2782*T + 0.1916*S + 22.6323*P + 0.00552*N - 0.0163*T*S - 0.0478*T*P + 0.00136*T*N + 0.0893*S*P + 0.00205*S*N - 0.0186*P*N - 0.00564*T^2 - 0.0266*S^2 - 3.8047*P^2 - 0.0000443*N^2 + 0.000606*T*S*P - 0.0000466*T*S*N - 0.00031*T*P*N - 0.000825*S*P*N + 0.000128*T*S^2 + 0.00000011*T*N^2 + 0.000291*T*P^2 + 0.000202*T^2*S - 0.0000004*T^2*N + 0.000718*T^2*P - 0.0129*S*P^2 + 0.0000043*S*N^2 + 0.00141*S^2*P + 0.00000212*S^2*N + 0.0000294*P*N^2 + 0.00273*P^2*N - 0.0000161*T^3 + 0.000396*S^3 + 0.2225*P^3 - 0.00000017*N^3 + 0.000016*T*S*P*N$$

$$R^2 = 0.843$$

Nonlinear data set:

$$\text{LN}(t_{4-D}) = -24.4041 + 0.0936*T + 0.1782*S + 13.0666*P - 0.0271*N - 0.00573*T*S - 0.0356*T*P + 0.00183*T*N - 0.0148*S*P + 0.00408*S*N - 0.0184*P*N - 0.000926*T^2 - 0.00854*S^2 - 1.4967*P^2 + 0.000152*N^2 - 0.00146*T*S*P - 0.0000846*T*S*N - 0.000478*T*P*N - 0.00128*S*P*N + 0.00013*T*S^2 - 0.00000038*T*N^2 + 0.00881*T*P^2 + 0.000143*T^2*S + 0.00000642*T^2*N - 0.00069*T^2*P + 0.00669*S*P^2 + 0.00000153*S*N^2 + 0.0000812*S^2*P + 0.00000805*S^2*N + 0.00000609*P*N^2 + 0.0037*P^2*N + 0.00000381*T^3 - 0.0000478*S^3 + 0.0306*P^3 - 0.00000025*N^3 + 0.0000279*T*S*P*N$$

$$R^2 = 0.865$$

* Based on natural logarithm transformation of t_{4-D} values generated using linear and nonlinear primary models to fit survivor curve data. Separate sets of models were generated using % lactic acid and pH as variables. (T = °C, S = % NaCl, L = % lactic acid, N = $\mu\text{g/mL}$ NaNO_2 , P = pH).

DISCUSSION

THE PRIMARY lethal factor causing inactivation of *L. monocytogenes* was the lactic acid. This represents the integration of two effects; the rate of inactivation of *L. monocytogenes* is dependent on both the adverse pH and an anion effect associated with the identity and concentration of the organic acid (Buchanan et al., 1993). Increasing the concentration of lactic acid (decreasing pH) accelerated inactivation as reflected in a decrease in D-values and either a decrease or elimination of t_L . Similar findings have been reported for *L. monocytogenes* (Sorrells et al., 1989; Cole et al., 1990; Ahamad and Marth, 1989, 1990; Buchanan et al., 1993), *Yersinia enterocolitica* (Little et al., 1992), and *Aeromonas hydrophila* (Palumbo and Williams, 1992). The pattern of inactivation is different from that observed by Parish and Higgins (1989) for the survival of *L. monocytogenes* in orange serum acidified with HCl. They reported that D-values were relatively independent of pH, whereas the lag period (i.e., t_L value) was decreased as pH was lowered.

Acid tolerance of *L. monocytogenes* was influenced by cultural history (Table 2), with survival enhanced by acidification of the starter cultures or addition of glucose to foster acid production. Kroll and Patchett (1992) reported that growing *L. monocytogenes* at pH 5.0 greatly enhanced its ability to survive subsequent exposure to pH 3.0 as compared to cells initially cultured at pH 7.0. This type of acid tolerance response (ATR) has been studied extensively for *Salmonella typhimurium* (Foster, 1991, 1992; Foster and Hall, 1990, 1991), and contributes to the survival of the pathogen in cheeses (Leyer and Johnson, 1992). Foster (1992) hypothesized that enhanced acid tolerance resulting from the growth of *S. typhimurium* in mildly acidic conditions was the result of an inducible pH homeostasis system which functions to maintain the intracellular pH. Viability was associated with maintaining an intracellular pH above 5.0—

5.5. However, Ita and Hutkins (1991) concluded that the inhibition of *L. monocytogenes* by organic acids was not the direct result of a drop in intracellular pH, but instead was related to specific effects of the undissociated acid on metabolic activities. Growth of *Staphylococcus aureus* in an unbuffered medium containing glucose influenced the accessory gene regulator which affects expression of many other genes, including several associated with virulence (Regassa et al., 1992). Farber and Pagotto (1992) reported that acid shock increased the thermal resistance of *L. monocytogenes*. It is apparent that conditions under which an organism is cultured can greatly influence its subsequent survival in an adverse environment. Our study employed culture conditions that fostered maximal acid tolerance of the inocula, thereby ensuring that models would be based on the pathogen being present in its resistant form.

While cultural history can influence the ability of *L. monocytogenes* to survive exposure to acidic conditions, continual or cyclic exposure to adverse conditions does not appear to lead to development of a population that is genetically more resistant (Fig. 3). Chung and Goepfert (1970) concluded that salmonellae could not be adapted to grow at lower pH levels by sequential exposure to a low pH environment; however, Huhtanen (1975) reported enhanced acid tolerance by repeated culturing on pH gradient plates.

Inactivation rates were influenced to varying degrees by the other environmental parameters. The most notable was temperature, with survival enhanced significantly by a reduction in temperature. The temperature dependent nature of acid-mediated inactivation of *L. monocytogenes* has been reported (Ahamed and Marth, 1989, 1990; Parish and Higgins, 1989; Sorrells et al., 1989; Cole et al., 1990; Conner et al., 1990; Sorrells and Enigl, 1990; Cherrington et al., 1992). While sodium nitrite has been reported to inhibit the growth of *L.*

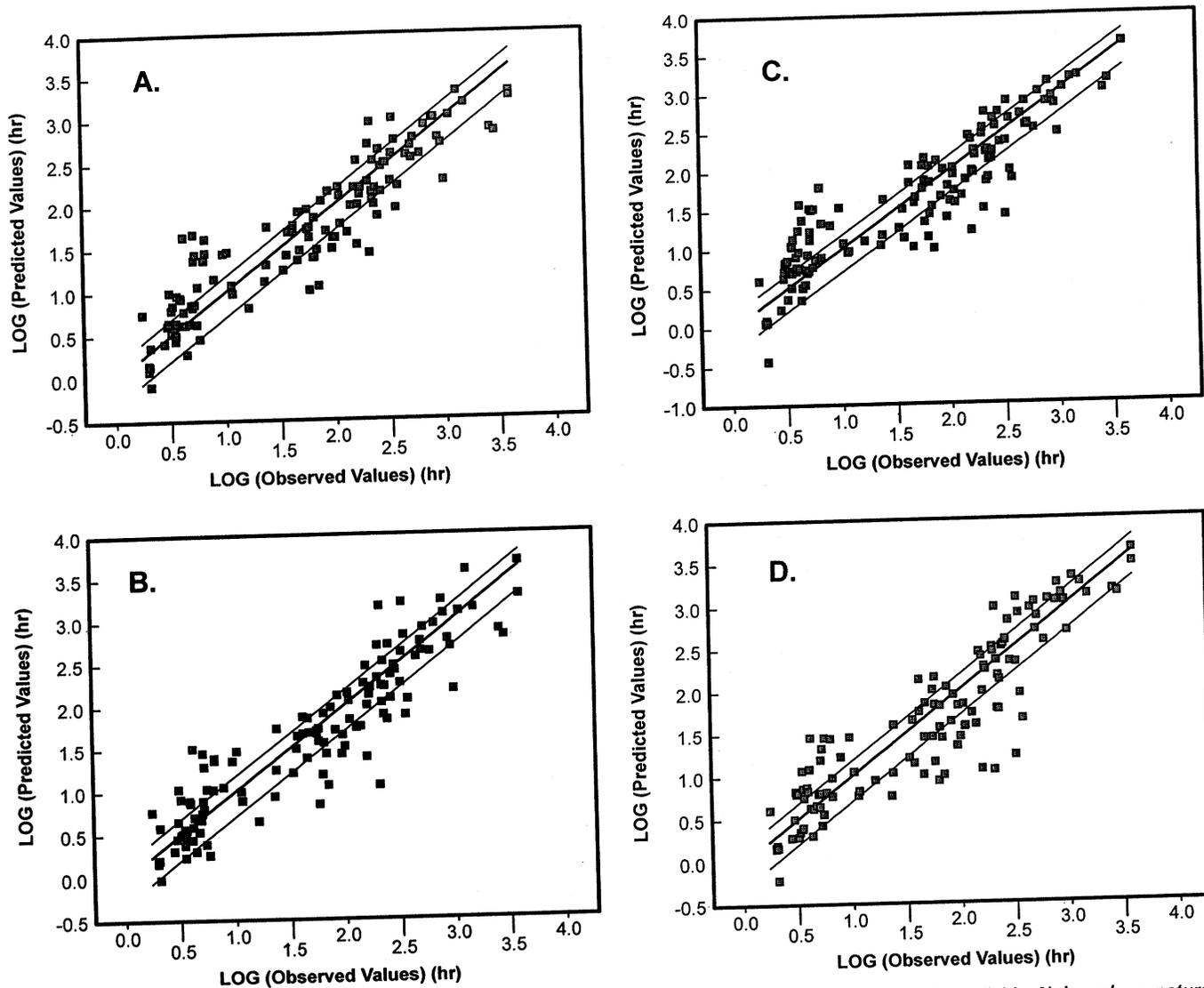


Fig. 4—Comparison of observed vs predicted t_{4-D} values for the four cubic response surface models (Table 3) based on natural logarithmic transformations of the t_{4-D} values generated using linear and non-linear primary models. Center line is the line of identity and the outer lines represent $\pm 50\%$ of the observed value. (A) Linear/Lactic acid, (B) Nonlinear/Lactic acid, (C) Linear/pH, and (D) Nonlinear/pH.

Table 4—Response surface models generated by submitting cubic models (Table 3) for the time for a 4-D inactivation of *Listeria monocytogenes* to backward regression analysis^a

Models based on using lactic acid as a variable:

Linear data set (Model 5):

$$\text{LN}(t_{4-D}) = 8.9354 + 0.0952*T - 5.7174*L - 0.0108*T*S - 0.0511*L*N - 0.00401*T^2 + 3.6059*L^2 + 0.0000835*L*N^2 + 0.000219*T^2*S + 0.0136*L^2*N - 1.0113*L^3 + 0.0000318*T*S*L*N$$

$$R^2 = 0.857$$

Nonlinear data set (Model 6):

$$\text{LN}(t_{4-D}) = 9.4818 - 5.4052*L + 0.08*S*L - 0.0664*L*N - 0.00292*T^2 - 0.0006*S^2 + 2.9112*L^2 + 0.000125*L*N^2 + 0.0000931*T^2*S + 0.000607*T^2*L + 0.016*L^2*N - 0.00429*T*S*L + 0.000458*T*L*N - 0.7928*L^3$$

$$R^2 = 0.878$$

Models based on using pH as a variable:

Linear data set (Model 7):

$$\text{LN}(t_{4-D}) = -29.61 + 18.2871*P + 0.000276*T*N - 0.0124*P*N - 0.00192*T^2 - 0.0032*S^2 - 2.908*P^2 + 0.0000147*P*N^2 + 0.00116*P^2*N + 0.1543*P^3$$

$$R^2 = 0.816$$

Nonlinear data set (Model 8):

$$\text{LN}(t_{4-D}) = -19.6507 + 8.9638*P - 0.0159*T*P + 0.000345*T*N - 0.0188*P*N - 0.00304*S^2 - 0.7579*P^2 + 0.000103*N^2 + 0.00346*T*P^2 - 0.000464*T^2*P + 0.00199*P^2*N$$

$$R^2 = 0.843$$

^a (T = °C, S = % NaCl, L = % lactic acid, N = $\mu\text{g}/\text{mL}$ NaNO₂, P = pH).

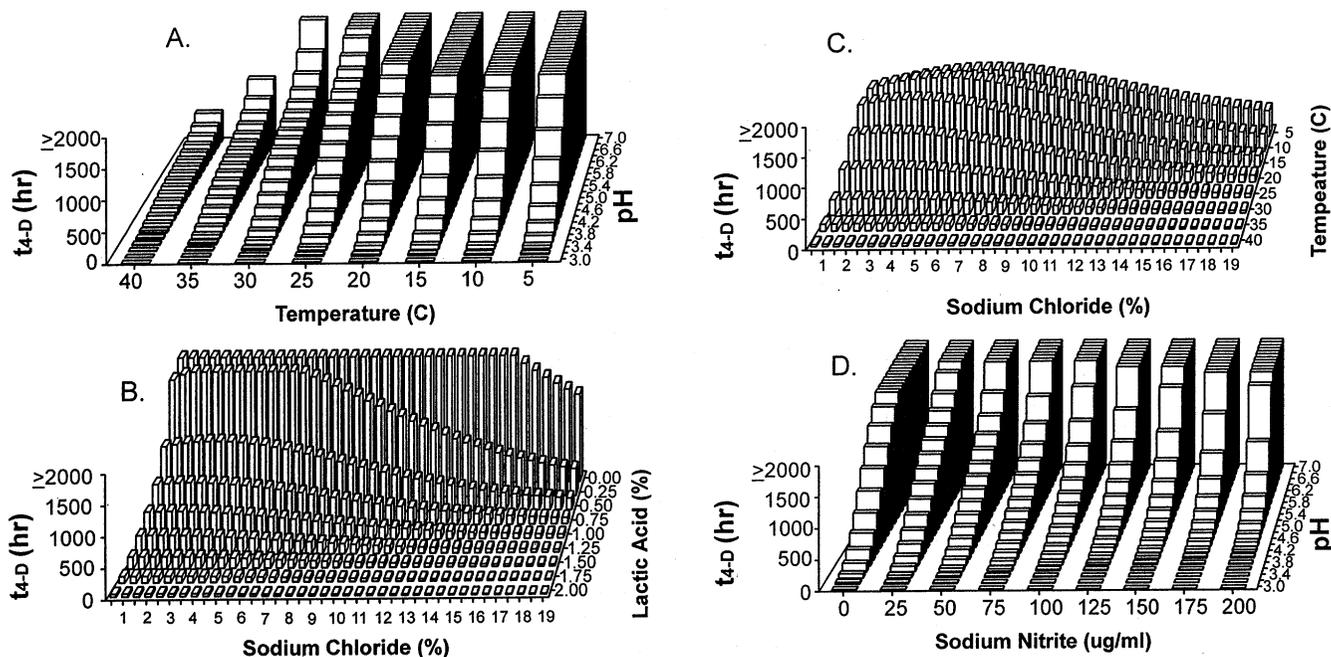


Fig. 5—Examples of the characteristics of the cubic response surface models based on the linear data set (Table 3, models 1 and 3). (A) temperature vs pH with NaCl = 0.5% and NaNO₂ = 0 µg/mL (model 3); (B) sodium chloride vs lactic acid with temperature = 15°C and NaNO₂ = 0 µg/mL (model 1); (C) temperature vs sodium chloride with lactic acid = 0.5% and NaNO₂ = 0 µg/mL (model 1); (D) pH vs sodium nitrite with temperature = 20°C and NaCl = 0.5% (model 3).

monocytogenes (Shahamat et al., 1980; Buchanan et al., 1989b; Buchanan and Phillips, 1990), this is one of the first studies to demonstrate that it can significantly influence the rate of inactivation. Junttila et al. (1989) reported that nitrite enhanced inactivation of *L. monocytogenes* in fermented sausages. The relatively minor effect of sodium chloride on inactivation of *L. monocytogenes* we observed confirms Hudson (1992) who reported that 16% NaCl did not affect survival over a 33-day incubation at -18°, 2° or 10°C. A slow rate of inactivation was noted when the NaCl concentration was increased to 26%. Cole et al. (1990) reported that low levels of NaCl provided a slight protective effect at low pH, while high NaCl levels enhanced inactivation. The relative insensitivity of *L. monocytogenes* to NaCl-mediated inactivation was expected considering its growth in the presence of relatively high NaCl concentrations (Buchanan et al., 1989b; Miller, 1992), presumably due to its ability to accumulate potassium and glycine betaine (Patchett et al., 1992).

The linear and logistics primary models for quantifying the inactivation curves had a high degree of agreement for calculated t_L , D/D_1 , and t_{4-D} values (Table 1). No systematic bias was apparent with t_{4-D} values derived from the two equations. While the nonlinear model provides a means for quantifying the inactivation rate of a more resistant subpopulation, no attempt was made to model this further. This decision was based on the inability to demonstrate the existence of a genetically stable resistant subpopulation (Fig. 3). Further, the effects of tailing did not generally become significant until there was >10,000-fold (i.e., 4-D) inactivation; the criterion that was selected as being a practical inactivation target for food products. The secondary models derived from the linear and logistics primary model data sets were generally similar in relation to fitting the experimental data (Fig. 4). However, overall the two models derived from the linear data had the best characteristics.

The development of an effective secondary model for describing the impact of environmental variables on nonthermal inactivation has some inherent limitations which must be overcome. The most obvious is the relatively large variability resulting, in part, from the extreme range of values encompassed. Depending on severity of conditions, inactivation rates ranged

from complete inactivation within several hours to no change in population densities after several months (Table 1). This was compounded by the presence of a t_L with a limited number of variable combinations. The approach employed to overcome these limitations was modeling the time to achieve a set decline in population density (t_{4-D}) and developing a large database so that the impact of the variability could be estimated. Model development was simplified by determination that, at least within the inoculum levels tested, kinetics of inactivation were independent of initial population density (Fig. 2).

An integral step in model development is validation, wherein the model is used to predict previously untested values. In part, this was incorporated into the experimental design which included four iterations of data acquisition and model generation and assessment. The models based on the linear data sets (models 1 and 3) were considered the most promising, providing predicted t_{4-D} values that were consistent with the pattern of inactivation kinetics observed by various investigators for *L. monocytogenes*. Figure 5 provides four examples of model 1 and 3's responses to changing variable combinations: temperature + pH (model 3) (Fig. 5A), sodium chloride + lactic acid (model 1) (Fig. 5B), temperature + sodium chloride (model 1) (Fig. 5C) and sodium nitrite + pH (model 3) (Fig. 5D). The temperature dependent nature of *L. monocytogenes* survival in an adverse acidic environment was described effectively, including a prediction that maximal survival should occur at about 10 to 12°C (Fig. 5A). This is in confirmation of several investigations which reported maximal survival in that temperature range (Ryser and Marth, 1987; Ahmad and Marth, 1989). The model also predicts that *L. monocytogenes* is relatively insensitive to sodium chloride at concentrations that would be encountered in foods (Fig. 5B and C); a characteristic reported by Hudson (1992) and Miller (1992). Intermediate levels of NaCl were somewhat protective, as reported by Cole et al. (1990). The models (Fig. 5D) also indicated that the activity of sodium nitrite is highly dependent on the pH/lactic acid concentration of the system.

The linear-based models were further evaluated by comparing their predictions with t_{4-D} values for different food and media systems (Table 5). These estimates were calculated using reported *L. monocytogenes* inactivation data. These compari-

Table 5—Comparison of $t_{4.0}$ values calculated from literature reports on the non-thermal inactivation of *Listeria monocytogenes* in food and microbiological media with those predicted by the linear response surface models [(Table, Model 1 (Lactic Acid) and Model 3 (pH))]

Product	Temp (C)	NaCl (%)	NaNO ₂ (µg/mL)	pH	Lactic acid (%)	Strain	$t_{4.0}$ Values (hr)			Reference	Comments
							Estimated from literature	Predicted			
								Model 1	Model 3		
Unclarified Cabbage Juice	4	0.5	0	5.6	NR*	Scott A	24,048	—	3,589	Conner et al. (1986)	Value of 0.5% assumed for samples with no supplemental NaCl. Extended literature values based on extrapolation of survivor curve.
	4	0.5	0	5.6	NR	LCDC81-861	3,456	—	3,589		
	4	1.0	0	5.6	NR	Scott A	24,048	—	3,991		
	4	1.0	0	5.6	NR	LCDC 81-861	3,340	—	3,991		
	4	4.5	0	5.6	NR	Scott A	6,336	—	6,720		
4	4.5	0	5.6	NR	LCDC 81-861	2,016	—	6,720			
Clarified Cabbage Juice	4	0.5	0	5.6	NR	LCDC 81-861	7,996	—	3,589	Conner et al. (1986)	*Growth followed by inactivation. Value of 0.5% assumed for samples with no supplemented NaCl.
	30	0.5	0	5.6	NR	LCDC 81-861	238*	—	857		
	4	0.5	0	4.8	NR	LCDC 81-861	2,667	—	1,666		
	30	0.5	0	4.8	NR	LCDC 81-861	336	—	604		
	4	0.5	0	4.4	NR	LCDC 81-861	202	—	885		
30	0.5	0	4.4	NR	LCDC 81-861	110	—	397			
Tryptic Soy Broth	35	10.0	0	4.8	NR	H4	613	—	225	Sorrells and Enigl (1990)	Based on estimates of initial and final populations only.
	25	12.0	0	4.8	NR	H4	870	—	684		
	10	10.0	0	5.2	NR	H4	2,800	—	4,177		
	10	8.0	0	5.0	NR	H4	4,556	—	3,934		
	35	10.0	0	4.8	NR	31C	646	—	255		
	25	12.0	0	4.8	NR	31C	1,050	—	684		
	10	10.0	0	5.2	NR	31C	1,687	—	4,177		
	10	8.0	0	5.0	NR	31C	1,200	—	3,934		
Tryptic Soy Broth + 0.6% Yeast Extract	4	0.5	0	4.5	NR	V-37, N7183 N7045, & N7095	1,048	—	1,061	Conner et al. (1990)	Value of 0.5% assumed for samples with no supplemented NaCl.
Tryptose Broth	13	0.5	0	NR	0.30	CA	748	1,708	—	Ahamad and Marth (1990)	
	13	0.5	0	NR	0.50	CA	516	1,094	—		
	35	0.5	0	NR	0.30	CA	75	173	—		
	35	0.5	0	NR	0.50	CA	52	109	—		
	13	0.5	0	NR	0.30	V7	748	1,708	—		
	13	0.5	0	NR	0.50	V7	548	1,094	—		
	35	0.5	0	NR	0.30	V7	77	173	—		
	35	0.5	0	NR	0.50	V7	62	109	—		
Salami	13	5.9	50	4.3	NR	Scott A	273	—	405	Johnson et al. (1968)	Held at 40 C for 24 hr, and then lowered to 13 C. Approximate 30% residual of initial 156 µg/g NaNO ₂ assumed.
Fermented Beaker Sausage	32	3.3	50	4.5	NR	Scott A, V7 LM101M, LM102M, & LM103M	124	—	131	Glass and Doyle, 1989	Initial pH 6.0, and fell to pH 4.8 within 12 hr during fermentation. Approximate 30% residual of initial 156 µg/g NaNO ₂ assumed.
Fermented Tea Sausage	18	2.5	30	4.6	NR	NTCC 7973	655	—	771	Buncic et al. (1991)	Initial pH 5.5, and fell to pH 4.8 during 4 day fermentation. Approximate 30% residual of initial 85 µg/g NaNO ₂ assumed.
Cheddar Cheese	6	1.4	0	5.1	NR	Scott A	6,284	—	2,909	Ryser and Martha (1987)	Approximate 1 log of growth before inactivation commenced.
	13	1.7	0	5.1	NR	Scott A	5,740	—	2,754		
	6	1.4	0	5.1	NR	V7	8,944	—	2,909		
	13	1.4	0	5.1	NR	V7	5,536	—	2,754		
Salami	11	3.3	70	5.0	NR	Serotype 1/2a	739	—	817	Trussel & Jemmi (1989)	pH decreased to 2.5 and NaCl increased to >5% by end of ripening period.
	11	3.3	20	5.0	NR	Serotype 1/2a	2,480	—	1,960		
Cottage Cheese	4	1.0	0	4.8	0.63	F6861	872	637	1,882	Hicks and Lund (1991)	Value for NaCl assumed. Also contained 0.11% citric acid. Also contained 0.07% citric acid.
	8	1.0	0	4.8	0.63	F6861	864	820	2,010		
	12	1.0	0	4.8	0.63	F6861	708	887	1,990		
	4	1.0	0	4.7	0.38	F6861	1,076	1,037	1,644		
	8	1.0	0	4.7	0.38	F6861	1,068	1,352	1,782		
	12	1.0	0	4.7	0.38	F6861	708	1,425	1,785		
	4	1.0	0	5.1	0.22	F6861	1,756	1,529	2,636		
12	1.0	0	5.1	0.22	F6861	2,708	2,213	2,577	Also contained 0.12% citric acid.		
Cole Slaw	4	0.5	0	5.0	NR	LM1	600	—	2,113	George and Levett (1990)	Value of 0.5% assumed for NaCl.
	4	0.5	0	6.0	NR	LM1	684	—	4,877		
	15	0.5	0	5.0	NR	LM1	720	—	2,067		
	15	0.5	0	6.0	NR	LM1	816	—	3,405		
25	0.5	0	5.0	NR	LM1	300	—	1,159			
Brain Heart Infusion Broth	28	12.6	0	7.4	0.00	Scott A	918	1,106	1,095	Miller (1992)	
	28	13.7	0	7.4	0.00	Scott A	584	981	927		
	28	16.7	0	7.4	0.00	Scott A	347	700	621		

* NR = Not reported.

sons were encouraging, demonstrating the feasibility of developing models that could be used to provide initial estimates of how long a product would need to be held to achieve a 4-D inactivation of *L. monocytogenes*. In general, use of pH as a variable (model 3) appeared more effective, likely reflecting that greater impact that pH had in comparison to acid concentration. Additional validation studies would be helpful to more rigorously assess the model's validity for a range of products, since several assumptions were made using available published data. Often variables had to be estimated or assumed if their values changed over time. For example, selection of variable values to substitute into the model was complicated for fermented sausages, since these meat products undergo substantial changes in pH, acidity, sodium chloride content, and water activity during manufacture and ripening. Several of the values for products (Table 5) involved extended survival of *L. monocytogenes*, and the D-values had to be calculated by extrapolation of limited microbiological data. These published values can only be considered estimates. Considering the limitations inherent in both the model and the validation data, the fits between predicted and reported values were quite reasonable.

Our study was undertaken to assess the feasibility of modeling non-thermal inactivation of foodborne pathogens, thereby allowing food manufacturers to predict the microbiological safety of processes and products. These initial results are encouraging, though they also identify several areas where additional research would greatly enhance effectiveness of future inactivation models. The most severe limitation of our models is the lack of independent terms for pH and lactic acid concentration. Survival of *L. monocytogenes* in an acidic environment is clearly dependent on pH, as well as the identity and concentration of the organic acid (Buchanan et al., 1993). Many foods can be buffered substantially, thereby confounding such models. The current models are for lactic acid only, and separate models must be developed for other organic acids. Other areas that need to be evaluated in future investigations include assessing effectiveness of the model for predicting inactivation under changing conditions, and determining if residual values are appropriate for estimating the impact of sodium nitrite. This latter area is particularly important in low pH systems where high levels of sodium nitrite greatly enhance inactivation. Not taking into account the disappearance of nitrite in food systems would result in an underestimation of *L. monocytogenes* survival. Data of this nature should enable development of improved models that should ultimately permit nonthermal inactivation processes to be estimated as effectively as currently done for thermal inactivation.

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