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# Predictive Thermal Inactivation Model for *Listeria monocytogenes* with Temperature, pH, NaCl, and Sodium Pyrophosphate as Controlling Factors†

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## ABSTRACT

The effects and interactions of heating temperature (55 to 65°C), pH (4 to 8), salt (NaCl; 0 to 6%, wt/vol), and sodium pyrophosphate (SPP; 0 to 0.3%, wt/vol) on the heat inactivation of a four-strain mixture of *Listeria monocytogenes* in beef gravy were examined. A factorial experimental design comparing 48 combinations of heating temperature, salt concentration, pH value, and SPP content was used. Heating was carried out using a submerged-coil heating apparatus. The recovery medium was plate count agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. Decimal reduction times (*D*-values) were calculated by fitting a survival model to the data with a curve-fitting program. The *D*-values were analyzed by second-order response surface regression for temperature, pH, NaCl, and SPP levels. Whereas increasing the NaCl concentration protected *L. monocytogenes* against the lethal effect of heat, high SPP concentrations increased heat sensitivity. Also, low pH values increased heat sensitivity of *L. monocytogenes*. The four variables interacted to affect the inactivation of the pathogen. Thermal resistance of *L. monocytogenes* can be lowered by combining these intrinsic factors. A predictive model that described the combined effect of temperature, pH, NaCl, and SPP levels on thermal resistance of *L. monocytogenes* was developed. The model can predict *D*-values for any combination of temperature, pH, NaCl, and SPP that are within the range of those tested. Using this predictive model, food processors should be able to design adequate thermal regimes to eliminate *L. monocytogenes* in thermally processed foods.

*Listeria monocytogenes,* the cause of listeriosis, is widespread in the environment *(21)* and has been found in raw and ready-to-eat vegetables, meat, poultry, seafood, and dairy products *(9, 10, 15, 35, 37, 43)*. Outbreaks of listeriosis involving a variety of contaminated foods have established *L. monocytogenes* as a psychrotrophic foodborne pathogen of major concern in the safety of refrigerated foods *(16, 27)*. In addition to illness and death, listeriosis causes significant economic losses, which in the United States have been estimated at 313 million dollars per year *(39)*. Based on the high mortality rate in the different outbreaks and on the lack of knowledge of the infectious dose for susceptible or immunocompromised individuals (such as neonates, cancer patients, the elderly, pregnant women, and those with AIDS or other illnesses that affect the immune system), the regulatory agencies in the United States specify a zero tolerance level for this contaminant in foods that do not receive further heat treatment prior to consumption *(11, 37)*.

One of the most common and effective means of controlling *L. monocytogenes* consists of the application of adequate heat treatment, followed by the adoption of prophylactic measures in order to prevent postheating recontamination of the cooked product *(37)*. However, *L. monocytogenes* control appears to be difficult because of the pathogen's relatively high tolerance for heat (compared with those tolerances of other nonsporeforming foodborne pathogens); in addition, postprocessing contamination with *L. monocytogenes* has been identified as a major source of contamination for many food products *(14)*. Mackey and Bratchell *(29)* indicated that *L. monocytogenes* appears to be appreciably more heat resistant than are *Salmonella* serotypes, but it appears to be less resistant than *Salmonella* Senftenberg 775W. Upon reviewing published heat-resistance data for *L. monocytogenes,* a decimel reduction time (*D*-)value of 2 min at 60°C and an average *z*-value of 6°C appears to be typical *(12, 29)*. Studies carried out on the heat resistance of *L. monocytogenes* have indicated that the composition of the heating menstruum can substantially influence thermal destruction of the organism. For example, the heat resistance of *L. monocytogenes* was greater in meat slurry than in phosphate buffer or milk *(1, 2)*. A processing temperature of 77°C for 16 s was adequate for pasteurization of dairy products *(17)*, but the organism survived in grilled meatballs that had been cooked to an internal temperature of 78 to 85°C for 15 min and in poultry products that had been exposed to cooking temperatures of 73.9 to 82.2°C *(20, 24)*. In food systems such as ground meats, thermotolerance can be increased with the addition of salt or curing-salt mixtures *(13, 30, 38, 45)*. Consequently, heat treatments must be designed to provide an adequate margin

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of safety against *L. monocytogenes* or any other foodborne pathogen.

Predictive modeling describes the behavior of microorganisms in foods by mathematically quantifying the effects of the interactions between two or more factors; further, predictive models allow interpolation of combinations that have not been tested explicitly *(44)*. These models aid in the formulation or reformulation of products with increased safety *(36)*. Whereas predictive growth kinetics models predict the time required for pathogens in food to reach dangerous levels under specific environmental conditions, thermal inactivation kinetics predictive models predict the target pathogen's survival within a specific range of food formulation variables. The latter models may help us to understand and to determine the extent to which existing/traditional thermal processes could be modified for a variety of cooked foods. Both growth and inactivation kinetics models enable food processors and regulatory agencies to ensure critical food-safety margins by predicting the combined effects of multiple food formulation variables.

There has been an urgent need for information concerning the effects of various intrinsic parameters in foods on thermal inactivation of *L. monocytogenes*. Accordingly, the aim of this study was to assess the effects and interactions of heating temperature, pH, salt (NaCl), and sodium pyrophosphate (SPP) on thermal inactivation of *L. monocytogenes* in a model system (beef gravy). The data were subsequently used to develop a four-factor thermal inactivation model that describes the combined effect of these factors in the heating menstruum on the thermal resistance of *L. monocytogenes*.

## MATERIALS AND METHODS

**Organisms.** The four strains of *L. monocytogenes* used throughout this study, Scott A, 20306, 418, and 201169, correspond to clinical, pheasant and pork pate, cooked poultry, and beef and pork frankfurter isolates, respectively. These strains, preserved by freezing the cultures at −70°C in vials containing tryptic soy broth (Difco, Detroit, Mich.), supplemented with 10% (vol/vol) glycerol (Sigma Chemical Co., St. Louis, Mo.), were obtained from our in-house culture collection.

**Culture preparation.** In order to propagate the cultures, vials were partially thawed at room temperature, and 1.0 ml of the thawed culture was transferred to 10 ml of brain–heart infusion broth (Difco) in 50-ml tubes and was incubated for 24 h at 37°C. The inoculum for use in heating tests was prepared by transferring 0.1 ml of each culture to 10-ml tubes of brain–heart infusion broth and by incubating aerobically for 24 h at 37°C in order to provide late stationary-phase cells. These cultures were maintained through consecutive daily transfers in tryptic soy broth for 2 weeks. Every 2 weeks, a new series of cultures from the frozen stock was initiated.

On the day of the experiment, each culture was pelleted by centrifugation (5,000 × *g*, 15 min, 4°C) and washed in 0.1% peptone water (wt/vol); this centrifugation and washing was repeated twice. The cell pellets were finally suspended in peptone water to a target level of 8 to 9 $\log_{10}$ CFU/ml. The population densities in each cell suspension were determined by spiral plating (Model D; Spiral Biotech, Bethesda, Md.) appropriate dilutions (in 0.1% peptone water), in duplicate, on tryptic soy agar (Difco) plates. Equal volumes of each culture were combined in a sterile test tube in order to obtain a four-strain mixture of *L. monocytogenes* (9 $\log_{10}$ CFU/ml) prior to inoculation of gravy.

**Beef gravy formulation.** The model beef gravy used in the present study consisted of 1.5% proteose peptone, 5.0% beef extract, 0.5% yeast extract, and 1.7% soluble starch. All ingredients were obtained from Difco Laboratories. NaCl (0.0 to 6.0%, wt/vol) and/or SPP (0.0 to 0.3%, wt/vol) was added to the beef gravy, which was again vortexed for 2 min to ensure even distribution. The pH of the gravy was adjusted to 4.0 to 8.0 using 85% (wt/wt) lactic acid (Sigma) and was determined using a combination electrode (Sensorex, semi-micro, A. H. Thomas, Philadelphia, Pa.) attached to an Orion Model 601A pH meter. The gravy was sterilized at 121°C for 15 min prior to use. After sterilization and equilibration to room temperature, the pH of the gravy was rechecked. If necessary, pH adjustments to the gravy were made with sterile solutions of the acid under a laminar-flow biological safety cabinet.

**Experimental design.** A fractional factorial design was employed in order to assess the effects and interactions of heating temperature (55, 57.5, 60, 62.5, and 65°C), NaCl (0.0, 1.5, 3.0, 4.5, and 6.0%; wt/vol), SPP (0.0, 0.1, 0.15, 0.2, and 0.3%; wt/vol), and pH (4.0, 5.0, 6.0, 7.0, and 8.0) levels. All 47 variable combinations were replicated twice.

**Beef gravy inoculation and thermal inactivation.** In sterile test tubes, beef gravy (10 ml) containing NaCl (0.0 to 6.0%, wt/vol) and SPP (0.0 to 0.3%, wt/vol) that was adjusted to various pH levels (4.0 to 8.0) was inoculated with 0.1 ml of the bacterial inoculum in order to obtain an initial concentration of approximately 7 to 8 $\log_{10}$ CFU/ml. Inoculated gravy samples were vortexed to ensure even distribution of the organisms. Thermal inactivation was carried out at 55 to 65°C using a submerged-coil heating apparatus *(7)*. This apparatus comprises a stainless-steel coil that is fully submerged in a thermostatically controlled water bath, which allows microbial suspensions to be heated to between 20 to 90°C with a short time to temperature equilibrium. During the heating procedure, samples (0.2 ml) were removed at predetermined time intervals. Where low cell numbers were expected, 0.6-ml aliquots were removed. Samples were rapidly cooled to room temperature in peptone water (0.1% wt/vol).

**Enumeration of surviving bacteria.** In order to determine the number of surviving CFU per milliliter after heat treatment, the beef gravy was serially diluted in 0.1% peptone water (wt/vol). Thereafter, appropriate dilutions were surface plated onto agar dishes containing tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate using a spiral plater. Also, 0.1 ml of undiluted suspension was surface plated, where relevant. All plates were incubated at 28°C for at least 48 h prior to enumeration of survivors. For each replicate experiment, an average surviving CFU of two platings of each sampling point was used to determine the *D*-values.

**Calculation of *D*- and *z*-values.** Regression lines were fitted to experimental data points that contributed to shouldering by a linear function (model) that allows for the presence of a lag period before initiation of an exponential decline in population density *(3, 4)*.

$$Y = Y_0 \qquad \text{for } T \leq T_L$$

$$Y = Y_0 + m(T - T_L) \qquad \text{for } T \geq T_L$$

where:

$Y$ = $\log_{10}$ count of bacteria at time $T$ [$\log_{10}$(CFU/ml)],

$Y_0$ = $\log_{10}$ count of bacteria at time $T = 0$ [$\log_{10}$(CFU/ml)],

$m$ = slope of the survivor curve [$\log_{10}$(CFU/ml)/min],

$T$ = time (min), and

$T_L$ = duration of lag period to initiation of inactivation (min).

The survivor curves were fitted using ABACUS, a nonlinear curve-fitting program that employs a Gauss–Newton iteration procedure *(8)*. *D*-values (time to inactivate 90% of the population) were calculated as the negative reciprocal of *m*.

The *z*-values (change in heating temperature needed to change the *D*-value by 90%) were estimated from the absolute value of the inverse slope by computing the linear regression *(31)* of mean $\log_{10}$ *D*-values versus their corresponding heating temperatures using Lotus 1-2-3 Software (Lotus Development Corporation, Cambridge, Mass.).

**Statistical modeling.** The *D*-values obtained were modeled as a function of the heating temperature, pH, NaCl, and SPP concentrations. After the *D*-values were transformed to the natural logarithm form in order to stabilize the variance of the response parameter, a quadratic response surface was used, which is represented by a polynomial of the following form:

$$\begin{aligned}\ln(y) = {} & c_1 + c_2\,(\text{temp}) + c_3\,(\text{pH}) + c_4\,(\text{salt}) + c_5\,(\text{phos}) \\ & + c_6\,(\text{temp})(\text{pH}) + c_7\,(\text{temp})(\text{salt}) \\ & + c_8\,(\text{temp})(\text{phos}) + c_9\,(\text{pH})(\text{salt}) + c_{10}\,(\text{pH})(\text{phos}) \\ & + c_{11}\,(\text{salt})(\text{phos}) + c_{12}\,(\text{temp})^2 + c_{13}\,(\text{pH})^2 \\ & + c_{14}\,(\text{salt})^2 + c_{15}(\text{phos})^2\end{aligned}$$

Within this polynomial, $\ln(y)$ is the natural logarithm of the dependent variable (i.e., the modeled *D*-value), and $c_1$ to $c_{15}$ are the coefficients to be estimated.

Thus, a four-factor response surface model was generated for curves fitted by the linear function. The coefficients were entered into a spreadsheet, and predictions of *D*-values were obtained within the range of experimental conditions of the heating menstruum.

## RESULTS AND DISCUSSION

This study determined the effects and interactions of temperature, pH, NaCl, and SPP levels in beef gravy on the inactivation of a four-strain *L. monocytogenes* cocktail. A mathematical model that predicts the *D*-value of this organism as a function of heating temperature, pH, NaCl, and SPP was developed. Based on a minimal root mean square value obtained using a linear model, the thermal inactivation data could be fitted well to generate survivor curves.

The coefficients obtained for the second-order polynomials were as follows:

$$\begin{aligned}\log_e D\text{-value} = {} & -61.4964 + 2.3019\,(\text{temp}) + 1.2236\,(\text{pH}) \\ & + 0.7728\,(\text{salt}) + 1.0477\,(\text{phos}) \\ & - 0.0102\,(\text{temp})(\text{pH}) - 0.0085\,(\text{temp})(\text{salt}) \\ & - 0.0566\,(\text{temp})(\text{phos}) - 0.0210\,(\text{pH})(\text{salt}) \\ & - 0.4160\,(\text{pH})(\text{phos}) + 0.1861\,(\text{salt})(\text{phos}) \\ & - 0.0217\,(\text{temp})^2 - 0.0273\,(\text{pH})^2 \\ & - 0.0213\,(\text{salt})^2 + 13.1605\,(\text{phos})^2\end{aligned}$$

The above multiple-regression equation for the $\log_e$ *D*-values yielded an $R^2$ value of 0.952. This equation is based on 47 unique combinations and can predict *D*-values/pathogen survival for any changes in the parameter values in the range tested from any combination of four environmental factors.

Table 1 shows the *D*-values of *L. monocytogenes,* based on survivor curves generated using the linear model, for the environmental variables of temperature, pH, NaCl, and SPP levels. The fit between *D*-values of *L. monocytogenes* in beef gravy, as predicted by the model, and those observed experimentally is depicted in Figure 1. Predicted *D*-values from the model compared well with the observed thermal death values. Thus, the model provides a valid description of the data used to generate it.

Mathematical models used to describe the heat destruction of microorganisms provide an opportunity to predict survival within a wide range of specific environmental conditions. In the traditional approach to establishing a safe thermal process, the rate of destruction of a microbial population is considered to follow first-order kinetics. This consideration is based on the assumption that bacterial cells within a population are identical. Significant deviations from the log-linear declines (logarithmic survivor curves), with characteristic lag periods or shoulders before any death occurs and a tailing, or a subpopulation of more resistant bacteria, that declines at a slower rate than the majority of the cells, have been observed *(23, 32, 40)*. Such deviations from the linear decline in the log number of survivors with time (i.e., sigmoidal curves) have been described with *L. monocytogenes.* A logistic equation *(5, 28)* or modified Gompertz equation *(6)* has been proposed to model such survivor curves. In the present study, the survivor curves fitted using a linear model showed an initial characteristic shoulder at 55°C. The "shoulder effect" observed may be attributed to the poor heat transfer through the heating menstruum and may be due to an initial requirement for the bacterial cells to sustain sufficient injury before the first-order inactivation kinetics in the log number of survivors with time. Another possible mechanism to explain this phenomenon is a distribution of heat resistance within the bacterial population *(18)*. *D*-values calculated from the linear portion of the survivor curves, while ignoring shoulders or lag periods, could lead to underestimation of the time and temperature needed to achieve a desired reduction in cell numbers. Therefore, in the present study, lag periods observed at 55°C were taken into account for calculating *D*-values. The nonlogarithmic survivor curves (sigmoidal curves) with characteristic shoulders and tailings are of significance in terms of public health, because such curves are likely to be observed in *sous-vide* processed foods, the microbiological safety of which relies on mild heat treatments and/or a combination of several inhibitory parameters (pH, water activity, etc.) at subinhibitory levels during food formulation. Researchers have expressed concerns about the microbiological risk involved in processing such new-generation food products *(19, 22)*.

Increasing acidity increased *L. monocytogenes* sensitivity to heat. For example, the predicted *D*-values at 55°C for

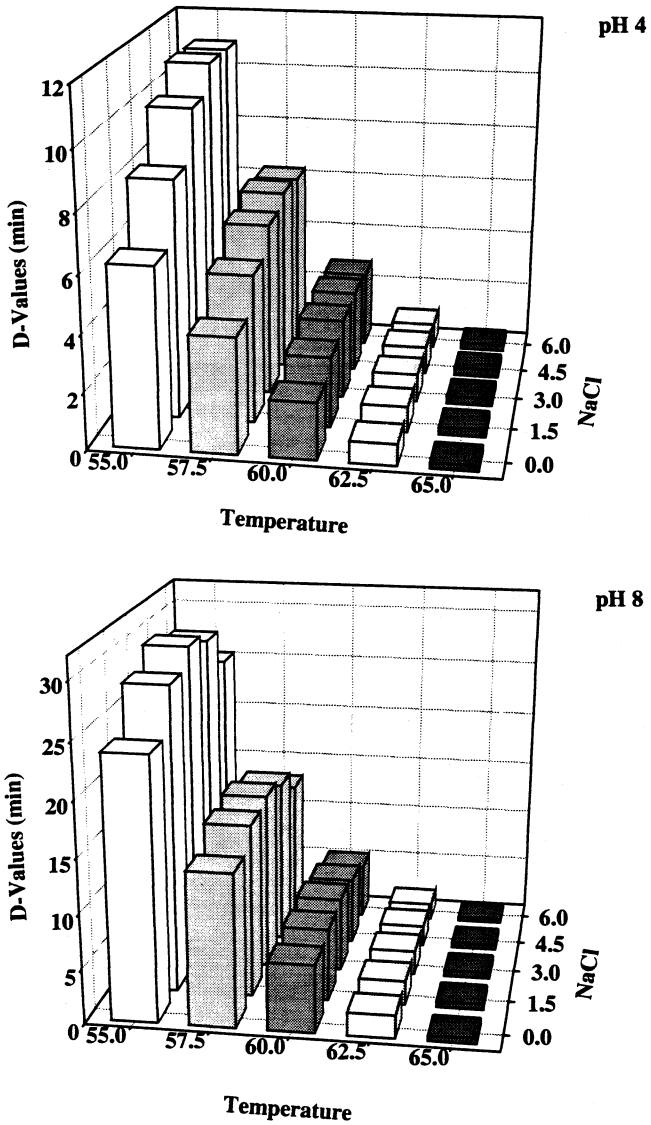


FIGURE 3. *Combined effect of NaCl and heating temperature on the predicted* D-*values of* L. monocytogenes *in beef gravy at pH 4 and 8.*

rate increases linearly in the acid and alkaline range and has a minimum at the optimum pH for growth.

NaCl protected against heat inactivation of *L. monocytogenes* in beef gravy at all temperatures (Fig. 3). When heating in gravy at low pH, an increase of up to 4.5% in NaCl concentration tended to decrease rates of thermal inactivation of *L. monocytogenes* at all temperatures (compared with control; i.e., in beef gravy with no NaCl present), but a concentration of 6% frequently led to a slightly more rapid inactivation of *L. monocytogenes*; however, the *D*-values were much higher than in the controls. A similar effect has been seen for *L. monocytogenes* at NaCl concentrations of up to 4.5% *(6)*. At higher pH levels in gravy (e.g., pH 8), the protective effect began to decline at NaCl concentrations of more than 3%. For example, the predicted *D*-values at 55°C increased from 23.87 min (gravy with no NaCl) to 27.95 and 29.72 min when the NaCl concentrations in gravy (pH 8) were 1.5 and 3.0%, respectively; the *D*-values were 28.71 or 25.20 min when 4.5 or 6% NaCl, respectively, was present in gravy. At other heating temperatures, the response of *L. monocytogenes* to NaCl levels in gravy was, in general, similar. Yen et al. *(45)* reported that the protective effect of NaCl against destruction of *L. monocytogenes* increased with increasing (0 to 3%) NaCl concentration. Overall, our findings concerning decreased destruction of *L. monocytogenes* in the presence of NaCl are in agreement with results of studies examining thermal destruction of the pathogen in broth, beef, and pork *(6, 13, 30, 45)*. In these studies, the *D*-values have been shown to increase two- to fivefold after curing salts were added to various blends of meat, whereby the concentration of NaCl in the final mixture was typically elevated to 3 to 4% (wt/wt). Therefore, the results of the present study can be used with confidence to predict the thermal destruction of *L. monocytogenes* in meat-based foods.

Several investigators have demonstrated that the thermal resistance of microorganisms is affected by salts in the heating menstruum *(25, 26)*. In these studies, the effects of salt on thermal resistance have mainly been examined by determining the relationships between thermal resistance and either solute concentration or water activity of the heating menstruum. Tuncan and Martin *(41)* suggested that the effect of salts on thermal inactivation of microorganisms is mainly related to reduced water activity and increased osmotic pressure of the heating menstruum. In a study by Reichart and Mohacsi-Farkas *(34)*, when heat destruction of seven foodborne microorganisms as a function of temperature, pH, redox potential, and water activity was assessed in synthetic heating media, the heat destruction increased with increasing water activity and decreasing pH. These results agree with those of the present study.

Figure 4 shows the effect of combinations of SPP concentration and heating temperature on *L. monocytogenes*. The lethality of heat to *L. monocytogenes* progressively increased with increasing SPP concentration up to 0.2% at all pH levels (4 to 8) of gravy. The predicted *D*-values at 55°C decreased (19.71%) from 6.24 min (gravy with 0% SPP) to 5.01 min in gravy with 0.2% SPP at pH 4 and from 23.87 to 13.74 min (42.44% decrease) at pH 8. At 0.3% SPP, the *D*-values slightly increased to 6.66 and 15.47 min in pH 4 and 8 gravy, respectively. A similar effect of SPP in the thermal destruction of *L. monocytogenes* was observed at higher heating temperatures (axiomatic) at all pH levels. These results do not agree with a previous study *(45)* in which phosphate combinations of 0.2% sodium tripolyphosphate and 0.2% sodium hexametaphosphate protected *L. monocytogenes* in pork from destruction during thermal processing. Yen et al. *(45)* reported that the destruction of *L. monocytogenes* was 0.8 log CFU/g less in ground pork with sodium phosphates than in ground pork alone. Contradictory results regarding the efficacy of sodium phosphates in altering the heat resistance of *L. monocytogenes* may be attributed to the heating menstruum. In the present study, we used beef gravy as the heating menstruum, as opposed to pork, which was used by Yen et al. *(45)*. In a study by Unda et al. *(42)*, the survival of *L. monocytogenes* in beef roasts that had been pumped with brine (containing phosphates and other ingredients) and cooked once or twice

TABLE 1. *Observed and predicted* D-*values at 55 to 65°C of* Listeria monocytogenes *in beef gravy, at various pH levels (4–8) supplemented with NaCl (0–6%, w/v), and sodium pyrophosphate (0–0.3%, w/v)*

| Temperature (°C) | pH | NaCl (%) | Phosphate (%) | *D*-value observed[a] | *D*-value predicted[b] |
|---|---|---|---|---|---|
| 55.0 | 4 | 0.0 | 0.00 | 5.35 (0.73) | 9.03 |
| 55.0 | 4 | 0.0 | 0.30 | 7.30 (0.79) | 9.62 |
| 55.0 | 4 | 6.0 | 0.00 | 12.49 (2.28) | 15.74 |
| 55.0 | 4 | 6.0 | 0.30 | 14.53 (0.36) | 24.45 |
| 55.0 | 6 | 3.0 | 0.15 | 12.88 (0.01) | 19.23 |
| 55.0 | 8 | 0.0 | 0.00 | 25.55 (2.64) | 35.13 |
| 55.0 | 8 | 0.0 | 0.30 | 21.64 (3.97) | 22.41 |
| 55.0 | 8 | 6.0 | 0.00 | 27.24 (0.78) | 36.52 |
| 55.0 | 8 | 6.0 | 0.30 | 18.61 (0.32) | 33.27 |
| 57.5 | 5 | 1.5 | 0.10 | 4.41 (0.08) | 6.53 |
| 57.5 | 5 | 1.5 | 0.20 | 6.06 (0.13) | 6.50 |
| 57.5 | 5 | 4.5 | 0.10 | 6.92 (0.38) | 8.05 |
| 57.5 | 5 | 4.5 | 0.20 | 10.61 (1.59) | 8.45 |
| 57.5 | 6 | 3.0 | 0.15 | 9.31 (1.32) | 9.11 |
| 57.5 | 7 | 1.5 | 0.10 | 5.83 (0.46) | 10.50 |
| 57.5 | 7 | 1.5 | 0.20 | 5.74 (0.35) | 9.59 |
| 57.5 | 7 | 4.5 | 0.10 | 10.42 (0.07) | 11.42 |
| 60.0 | 4 | 3.0 | 0.15 | 1.91 (0.17) | 3.04 |
| 60.0 | 5 | 3.0 | 0.15 | 4.39 (0.18) | 3.25 |
| 60.0 | 6 | 0.0 | 0.15 | 2.82 (0.14) | 3.45 |
| 60.0 | 6 | 1.5 | 0.15 | 2.51 (0.02) | 3.52 |
| 60.0 | 6 | 3.0 | 0.00 | 4.99 (0.21) | 6.76 |
| 60.0 | 6 | 3.0 | 0.10 | 4.18 (0.29) | 4.08 |
| 60.0 | 6 | 3.0 | 0.15 | 3.15 (0.12) | 3.83 |
| 60.0 | 6 | 3.0 | 0.20 | 3.15 (0.12) | 3.95 |
| 60.0 | 6 | 3.0 | 0.30 | 3.91 (0.04) | 6.10 |
| 60.0 | 6 | 4.5 | 0.15 | 4.34 (0.16) | 3.94 |
| 60.0 | 6 | 6.0 | 0.15 | 3.53 (0.23) | 4.30 |
| 60.0 | 7 | 3.0 | 0.15 | 4.33 (0.39) | 4.46 |
| 60.0 | 8 | 3.0 | 0.15 | 5.47 (1.16) | 5.76 |
| 62.5 | 5 | 1.5 | 0.10 | 0.78 (0.00) | 1.05 |
| 62.5 | 5 | 1.5 | 0.20 | 0.89 (0.01) | 1.01 |
| 62.5 | 5 | 4.5 | 0.10 | 1.40 (0.01) | 1.14 |
| 62.5 | 5 | 4.5 | 0.20 | 0.53 (0.02) | 1.16 |
| 62.5 | 6 | 3.0 | 0.15 | 3.53 (0.23) | 1.28 |
| 62.5 | 7 | 1.5 | 0.10 | 1.34 (0.04) | 1.59 |
| 62.5 | 7 | 1.5 | 0.20 | 1.40 (0.04) | 1.36 |
| 62.5 | 7 | 4.5 | 0.10 | 0.66 (0.03) | 1.46 |
| 62.5 | 7 | 4.5 | 0.20 | 0.63 (0.07) | 1.37 |
| 65.0 | 4 | 0.0 | 0.00 | 0.26 (0.01) | 0.32 |
| 65.0 | 4 | 0.0 | 0.30 | 0.17 (0.00) | 0.28 |
| 65.0 | 4 | 6.0 | 0.00 | 0.17 (0.01) | 0.33 |
| 65.0 | 4 | 6.0 | 0.30 | 0.33 (0.01) | 0.41 |
| 65.0 | 6 | 3.0 | 0.15 | 0.28 (0.01) | 0.38 |
| 65.0 | 8 | 0 | 0.30 | 0.24 (0.00) | 0.44 |
| 65.0 | 8 | 6 | 0.00 | 0.36 (0.00) | 0.51 |
| 65.0 | 8 | 6 | 0.30 | 0.37 (0.03) | 0.38 |

[a] Values represent means (standard deviations) of 47 variable combinations replicated twice.

[b] The upper limit of confidence interval of predicted *D*-value.

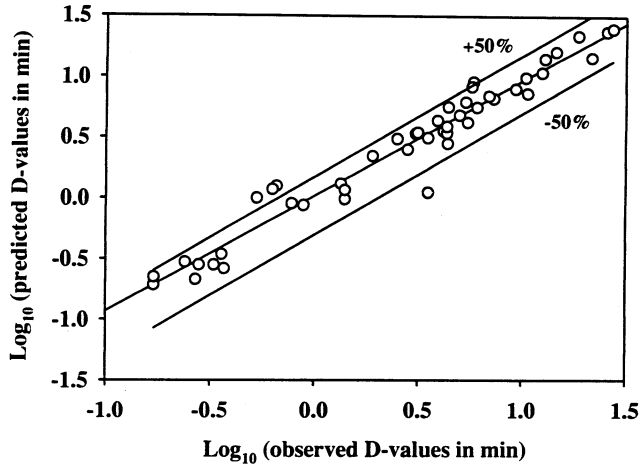


FIGURE 1. *Agreement between predicted and observed* D-*values of* L. monocytogenes *in beef gravy. The center line is the "line of identity" and the others represent ±50% of the observed value.*

gravy with no SPP or NaCl present were 6.24 and 23.87 min at pH 4 and 8, respectively (Fig. 2). Decreasing the pH from 8 to 4 resulted in a parallel decrease in predicted *D*-value by 73.86% at 55°C, with decreases of 71.07% at 57.5°C, of 68.06% at 60°C, of 64.29% at 62.5°C, and of 58.82% at 65°C (Fig. 2). Thus, the pH effect was observed at all heating temperatures. The pH of the heating menstruum is recognized as one of the most important factors influencing the heat resistance of bacteria. Microorganisms usually have their maximum heat resistance at pH values close to neutrality; a decrease in the pH of the heating medium usually results in a decreased *D*-value. Reichart *(33)* provided a theoretical interpretation of the effect of pH on heat destruction and described a linear relationship between pH and the logarithm of the *D*-values for *Escherichia coli.* The author stated that the logarithm of the heat-destruction

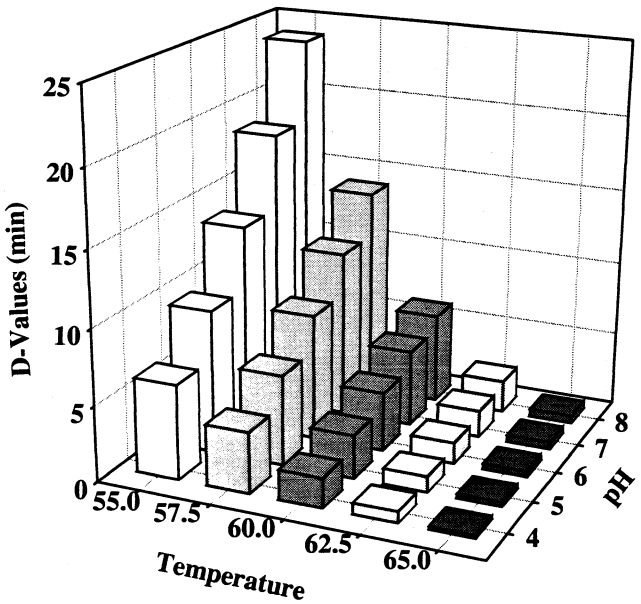


FIGURE 2. *Combined effect of pH and heating temperature on the predicted* D-*values of* L. monocytogenes *in beef gravy.*

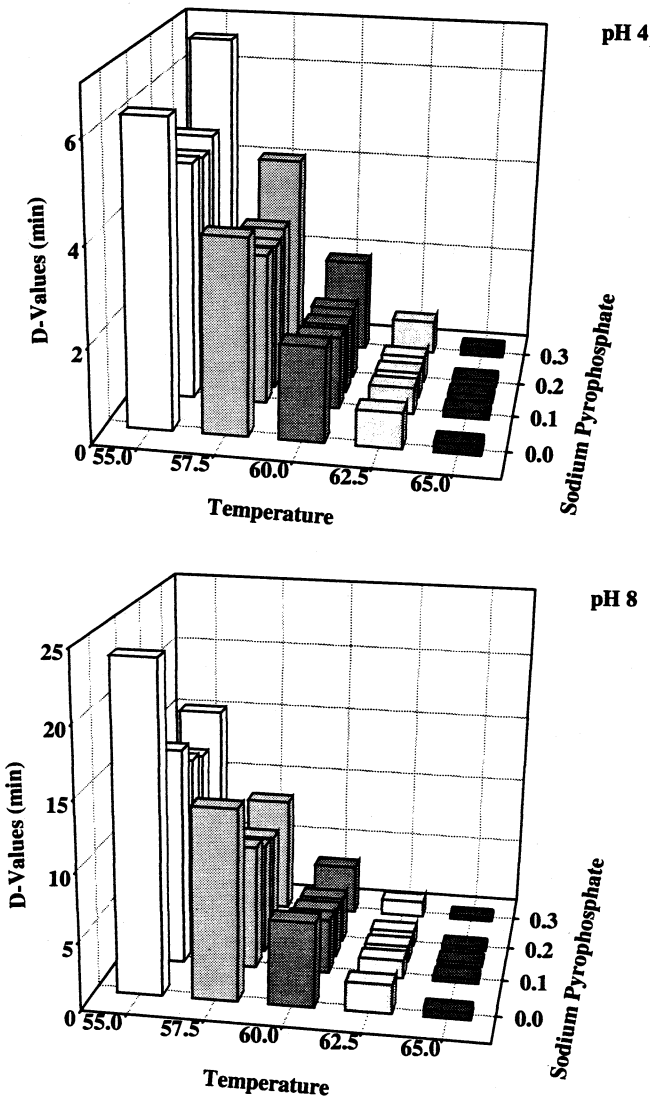


FIGURE 4. *Combined effect of SPP and heating temperature on the predicted* D-*values of* L. monocytogenes *in beef gravy at pH 4 and 8.*

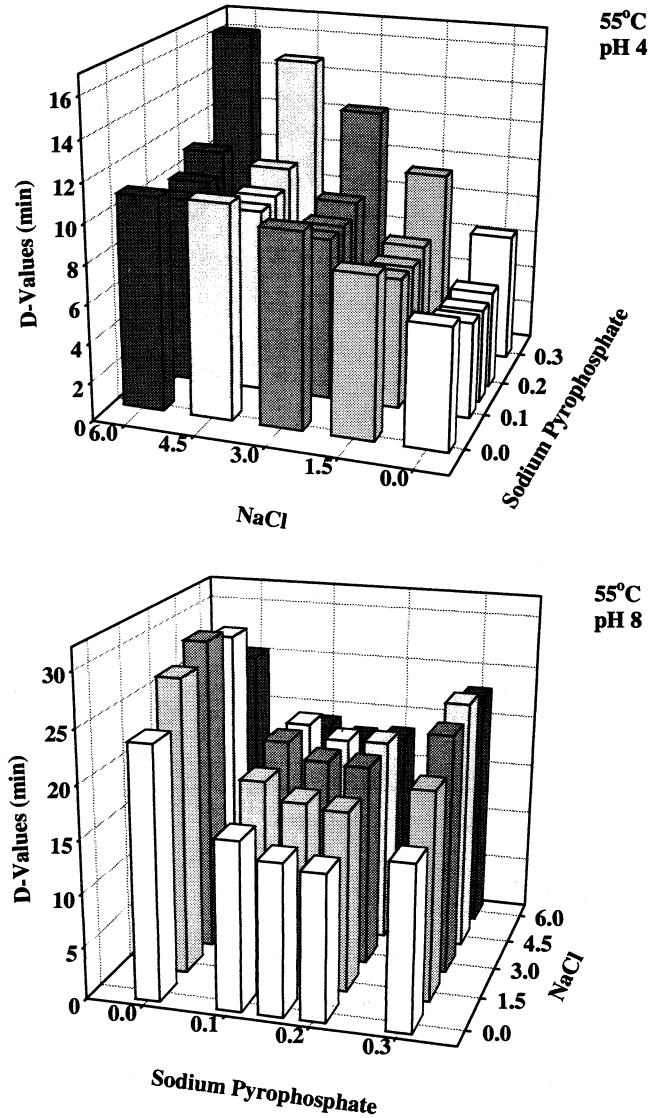


FIGURE 5. *Effects and interactions of NaCl and SPP on the predicted* D-*values, at 55°C, of four-strain mixture of* L. monocytogenes *in beef gravy at pH 4 and 8.*

to 62.8°C was reduced by the phosphate blend. However, the authors did not indicate whether the phosphate blend decreased the survival of *L. monocytogenes* during cooking or whether it inhibited the recovery of the survivors during cold storage.

Results showed that all variables (pH, NaCl, and SPP) affect *D*-values of *L. monocytogenes* at all heating temperatures studied. An example of the effects and interaction of 0 to 6% NaCl and 0 to 0.3% SPP in gravy at pH levels 4 and 8 on the predicted *D*-values is presented in Figure 5. Lower pH tended to increase heat sensitivity at 55°C. This effect was also observed when SPP was present in gravy. However, NaCl addition in gravy in the presence of SPP, regardless of pH level, slightly increased the degree of thermotolerance. For example, the predicted *D*-values at 55°C decreased (73.86%) from 23.87 to 6.24 min as the pH of the gravy decreased from 8 to 4. When heated in gravy containing 0.3% SPP, the *D*-values in pH 4 gravy was 56.95% of the value at pH 8 (6.66 versus 15.47 min); however, supplementing gravy with 1.5% NaCl increased *D*-values to 9.61 and 19.69 min in pH 4 and 8 gravy, respectively. The protective effect of NaCl in the presence of SPP confirmed previous findings *(13, 30)*. In a study by Farber et al. *(13)*, when heat resistance of *L. monocytogenes* in minced- and sausage-type meat at 58 to 62°C was assessed, *D*-values ranged from 1.01 min at 62°C to 6.39 min at 58°C and from 7.06 min at 62°C to 50.0 min at 58°C, respectively. Thus, the *D*-values obtained for ground meat plus cure (i.e., a mixture of nitrite, salt, dextrose, lactose, and corn syrup) were five- to eightfold higher than those for ground meat alone. Mackey et al. *(30)* compared heat resistance of *L. monocytogenes* in fresh and cured beef and chicken and derived an equation to predict the time necessary to achieve a "7D" inactivation at temperatures between 55 and 70°C, which at 70°C would be 1.45 min. In their study, addition of curing salt had a protective effect and enhanced the heat resistance. The results of our study are in agreement with previous studies *(13, 30)* and suggest

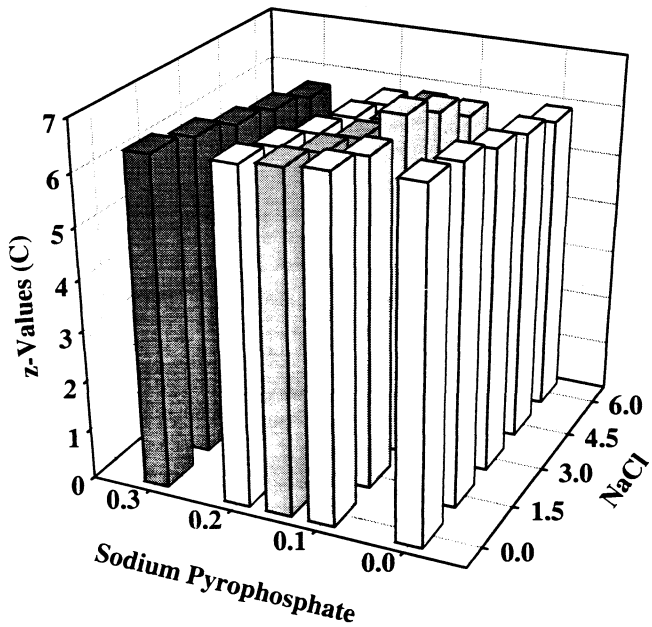


FIGURE 6. *The z-values of* L. monocytogenes, *calculated from predicted* D-*values obtained in beef gravy (pH 4) supplemented with 0.0 to 6.0% NaCl and/or 0.0 to 0.3% SPP.*

that solutes, such as salt, in processed meat may protect *L. monocytogenes* from heat injury.

The predictive relative impact of various levels of NaCl and SPP in increasing the sensitivity of *L. monocytogenes* to heat is shown in Figure 6. The *z*-value calculated from predicted *D*-values obtained in gravy (pH 4) with no added NaCl or SPP was 6.8°C. Presence of 1.5% NaCl or 0.1% SPP decreased *z*-values to 6.64 or 6.72°C, respectively. Increasing NaCl or SPP levels resulted in a parallel decrease in *z*-values. For example, the *z*-values were 5.91 or 6.48°C in the presence of 6% NaCl or 0.3% SPP, respectively. A combination of NaCl and SPP was more effective in lowering the *z*-values. The lower *z*-values in the presence of NaCl and SPP observed in this study confirmed the findings of Farber *(12),* who reported *z*-values of 4.92 and 3.5 in ground meat and ground meat plus cure, respectively. However, the lower *z*-values are not in agreement with the studies of Schoeni et al. *(38),* who observed *z*-values of 10°C in fermented beaker sausage compared with 7.9°C in ground beef roast. A possible explanation for the contradictory results of the various studies could be that certain strains of *L. monocytogenes* are less heat resistant and are thus less tolerant of changes in temperature. According to the present study, smaller changes in temperature are required to cause 90% reduction in *D*-value when a cocktail of *L. monocytogenes* strains is evaluated in gravy with increasing levels of NaCl and/or SPP. Certainly, it would not be logical to determine *z*-values under one set of food formulation variables and apply them to another set of parameters in foods.

In conclusion, a mathematical model, based on a multifactorial design experiment, that describes and predicts the effects and interactions of pH, NaCl, and SPP on the thermal inactivation of *L. monocytogenes* has been developed. Thermal resistance of *L. monocytogenes* can be altered by manipulating these intrinsic factors. The predictive model allows for a strong prediction of microbial survival under conditions different from those tested experimentally but within the experimental/studied range of parameters used to generate the data. The model can contribute to more effective evaluation and assessment of the impact of changes in food formulations on the heat lethality of *L. monocytogenes.* By using this predictive model, food processors should be able to design appropriate processing times and temperatures that can ensure protection against *L. monocytogenes* in processed meats.

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