APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1997, p. 1237–1243 Vol. 63, No. 4
0099-2240/97/$04.00+0
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Mathematical Models for the Effects of pH, Temperature, and Sodium Chloride on the Growth of *Bacillus stearothermophilus* in Salty Carrots†

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Received 16 August 1996/Accepted 16 January 1997

Estimating the shelf life and safety of any food product is an important part of food product development. Predictive food microbiology reduces the time and expense associated with conventional challenge and shelf life testing. The purpose of this study was to characterize and model germination, outgrowth, and lag (GOL) time and the exponential growth rate (EGR) of *Bacillus stearothermophilus* in salty carrot medium (SCM) as a function of pH, temperature, and NaCl concentration. *B. stearothermophilus* is a spore-forming thermophilic organism associated with flat sour spoilage of canned foods. A split-split plot design was used to measure the effects and interactions of pH (5.5 to 7.0), temperature (45 to 60°C), and NaCl (0 to 1%) on the growth kinetics of *B. stearothermophilus* in SCM. A total of 96 experiments were analyzed, with individual curve parameters determined by using the Gompertz equation. Quadratic polynomial models for GOL time and EGR of *B. stearothermophilus* in terms of temperature, pH, and NaCl were generated by response surface analysis. The r^2 values for the GOL time and EGR models were 0.917 and 0.916, respectively. These models provide an estimate of bacterial growth in response to combinations of the variables studied within the specified ranges. The models were used to predict GOL times and EGRs for additional experimental conditions. The accuracy of these predictions validated the model's predictive ability in SCM.

Bacillus stearothermophilus is a gram-positive, facultatively anaerobic, thermophilic, spore-forming organism. Two important characteristics of this bacterium are its abilities to form very heat-resistant spores and to grow at high temperatures (40 to 70°C).

Thermal processing is usually directed against mesophilic organisms and is not designed to eliminate thermophilic spore-formers such as *B. stearothermophilus* (17). If a canned food product containing viable *B. stearothermophilus* spores is stored at temperatures above 43°C, flat sour spoilage will likely result. Increasing the processing time and temperature to inactivate thermophilic spores would result in an overcooked and organoleptically unacceptable product. Feeherry et al. (13) noted that many food products cannot withstand the heat treatment needed to inactivate thermophilic spores. Thermophiles can be a serious problem, especially where foods must be stored at elevated temperatures for a long time (e.g., canned food vending machines or military operations in tropical climates).

Mathematical modeling techniques are gradually becoming recognized and accepted for estimating the shelf life and safety of many food products (30). The development of growth models allows the food microbiologist to get an accurate prediction of food quality or safety with speed and confidence (2). Many models for the growth of pathogens have been reported in the literature during the last decade, including models for *Aeromonas hydrophila* (25), *Clostridium botulinum* (15, 29), *Listeria monocytogenes* (7, 9, 19), *Salmonella* spp. (5, 11), *Shigella flexneri* (32), *Staphylococcus aureus* (8, 12), and *Yersinia enterocolitica* (4). However, there have been few reports on the predictive microbiology of food spoilage bacteria (24).

This research was designed to begin to increase our limited knowledge of the suitability of predictive food microbiology for solving food spoilage problems. Mathematical models for the effects of temperature, pH, and NaCl concentration on *B. stearothermophilus* exponential growth rate (EGR) germination, outgrowth, and lag (GOL) time in salty carrot medium (SCM) were developed. *Bacillus stearothermophilus* was chosen as a representative thermophile. SCM was used to conduct the experiments, because it is homogeneous and easy to prepare. NaCl and pH were chosen as variables, since these parameters can be manipulated fairly easily by the food processor. The resulting models should be a useful first step toward predicting the microbiological shelf life of various thermostabilized foods stored under high-temperature conditions.

MATERIALS AND METHODS

Type of strain used. The spores of *B. stearothermophilus* ATCC 12980 were used in this research.

Growth and harvesting of spores. Spores of *B. stearothermophilus* ATCC 12980 were produced on plates of tryptic soy agar (TSA) (Difco, Detroit, Mich.) with $MnSO_4$ (50 mg/liter), $MgSO_4$ (100 mg/liter), and $CaCl_2$ (500 mg/liter). The sporulation procedure was as follows. The plates were inoculated with a suspension of vegetative cells of *B. stearothermophilus* ATCC 12980 in tryptic soy broth and incubated for 48 h at 55°C. Cultures from the plates were harvested by scraping the agar surface with a loop and rinsing off the growth with sterile distilled water. Purification was achieved by centrifugation. The suspension was centrifuged at 10,000 × *g* for 10 min. The suspension was discarded, and the pellet was washed five times by suspension in sterile distilled water, followed by recentrifugation (2,500 × *g* for 15 min and 2,000 × *g* for 15 min; four times). Observation of the suspension under the microscope with phase optics showed >95% refractile spores and no vegetative cells. The pellets were subsequently suspended in sterile distilled water. Spores were counted by plating 0.5 ml of decimal dilutions on TSA medium and incubating at 55°C for 24 h. Suspended spores were then diluted into sterile water to obtain a spore concentration of 10^5 to 10^6 per ml. The spore suspensions were stored at 4°C for up to 6 months until use.

Preparation of SCM. Mixtures of 0, 0.5, and 1% brine salty carrots were made from water, NaCl, and carrots. These salty carrot mixtures were filled into

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† Publication D-10575-2-96 of the New Jersey Agricultural Experiment Station.

pouches, sealed, and retorted at 119.5°F for 13 min at the Center for Advanced Food Technology, Food Manufacturing Technology Facility. Salty carrots were homogenized in a sterile commercial blender (Waring Products) for 10 min. The pH values were adjusted with hydrochloric acid and sodium hydroxide. Nine milliliters of SCM was placed into sterile tubes prior to inoculation with the spore suspension.

Experimental methods. Spores were activated by heating in a boiling-water bath at 100°C for 10 min. The activated solution was used to inoculate the SCM to give a final concentration between 10^1 and 10^2 CFU/ml. The tubes were placed in a digital water bath (Fisher Scientific, Pittsburgh, Pa.) preset at the desired temperature. Samples were taken from the water bath and diluted at given time intervals. Dilutions were made in Butterfield phosphate buffer ($MnSO_4$ [50 μg/liter], $MgSO_4$ [10 ml/liter], $CaCl_2$ [1 ml/liter]). TSA plates were poured in duplicate at appropriate dilution levels. The plates were incubated at 55°C for 24 h in a bench top incubator (model 146A; Fisher) before the number of CFU per milliliter were determined. Duplicate plates showing a suitable number of colonies (25 to 250) were counted with a Darkfield Quebec Colony Counter (Reichert-Jung, Buffalo, N.Y.). A "no-growth" determination was made if the number of microbes continuously declined over time to a nondetectable level (<21 CFU/ml) (3).

Experimental design. A split-split plot design (18) with three independent variables was used in this experiment. The three variables were NaCl concentration (0, 0.5, and 1%), pH (5.5, 6.0, 6.5, and 7.0), and temperature (60, 55, 50, and 45°C). The response variables were GOL time and EGR. There are two split-split plot randomization restrictions, since the NaCl level is chosen first, then the pH value is chosen, and then the temperature is randomized for that particular NaCl and pH combination. The entire experimental design was repeated twice. The three concentrations of NaCl and two replications form the whole plots. The pH levels are randomized at each concentration of NaCl and in each replication to form a split plot. Then, at each pH-NaCl-replication combination, the four levels of temperature are randomly applied, forming what is called a split-split plot, indicating that two main effects (NaCl and pH) are confounded with blocks. A split-split plot design was chosen over a factorial design, because the former offers fewer medium formulation steps (23). Ninety-six experiments were run in this investigation.

Additional validation experiments at different temperatures and pH values were collected by the methods discussed above. Validation conditions were chosen based on typical values one might encounter in high-temperature spoilage of canned foods (pH, 5.75 to 7.00; temperature, 46 to 50°C; NaCl, 0.5%).

Curve fitting and model development. Plate counts were transformed to $\log_{10}$ values, and growth curves were determined by fitting the Gompertz function to the plate count data with Genstat 5.22 (Numerical Algorithms Group, Downers Grove, Ill.).

The Gompertz equation is as follows:

$$\log(N_t) = \log(N_0) + C \cdot e^{-e^{[-B(t-M)]}} \quad (1)$$

where t is the time (in hours) since inoculation; N_t and N_0 are the population densities (in CFU per milliliter) at time t and time zero, respectively; C is the logarithm of the population change from inoculation to the stationary phase; B is the relative growth rate (dimensionless) at M; and M is the time (in hours) at which the growth rate is at a maximum. The growth kinetic equations were determined from the above-defined parameters, as follows: GOL time $= M - 1/B$ and EGR $= BC/e$.

The Gompertz parameters were used to predict the GOL time and EGR with SAS software (SAS Institute, Cary, N.C.). Conditions in designed experiments used in predictive food microbiology may be such that bacteria fail to grow. Analysis of designed experiments requires that a judgment be made regarding missing data. When the bacteria failed to grow, EGR was reported to be zero, i.e., no growth. EGR could have been reported as "missing data," rather than zero, but this would result in a possible overestimation of predicted growth rate for some conditions. The situation with GOL time is somewhat different. No growth means infinite GOL time, but it is impossible to represent infinity in the analysis. The only solution, in this case, is to represent the missing data as such. The predictions for GOL time for no-growth conditions rely on extrapolations of GOL times from conditions where growth did occur and are therefore less accurate than EGR predictions for the same conditions.

Analysis of variance procedures (18) were used to determine GOL time and EGR differences for NaCl, pH, and temperature treatments. The least significant difference test (18) was used to determine interactions when analysis of variance indicated significant differences ($\alpha = 0.05$).

The Box-Cox (26) method was used to determine which transformation best decreased the variance of the prediction. The Box-Cox method determines the value of lambda (the transformation power) which maximizes r^2. This lambda is the "perfect" transformation of the data.

Quadratic polynomial (response surface) models for the effects of pH, temperature, and NaCl on transformations of the GOL time and EGR values were generated with SAS software. Backward stepwise (BS) regression was used to eliminate the parameters which were not significant in the model. The lack-of-fit test was used to determine whether the full polynomial model or the simplified polynomial model was a more appropriate fit of the experimental data.

TABLE 1. Effect of culture conditions on the calculated average values of the Gompertz parameters on the EGR and GOL time for the growth of *B. stearothermophilus*

pH	Temp (°C)	Value for EGR or GOL time at indicated NaCl concn[a]					
		0.0%		0.5%		1.0%	
		EGR	GOL time	EGR	GOL time	EGR	GOL time
5.5	45	0.00	—	0.00	—	0.00	—
	50	0.27	25.2	0.34	14.1	0.14	28.9
	55	0.23	19.8	0.60	6.9	0.30	11.1
	60	0.15	17.6	0.45	6.8	0.49	12.7
6.0	45	0.00	—	0.25	11.6	0.00	—
	50	0.21	12.6	0.50	7.5	0.34	8.3
	55	0.36	5.3	0.61	5.1	0.50	5.3
	60	0.34	5.9	0.66	5.7	0.58	7.2
6.5	45	0.00	—	0.21	13.0	0.19	12.9
	50	0.32	7.2	0.43	4.8	0.34	7.3
	55	0.68	5.8	0.84	3.3	0.82	5.4
	60	0.71	6.6	0.85	3.3	0.62	4.6
7.0	45	0.20	11.9	0.31	9.3	0.26	11.4
	50	0.42	4.9	0.73	4.8	0.50	7.3
	55	0.58	3.3	1.02	2.5	0.69	3.4
	60	0.61	3.5	0.97	2.5	0.82	4.0

[a] Data for EGR and GOL time are reported in hour^{-1} and hours, respectively. —, missing data.

RESULTS AND DISCUSSION

GOL time, EGR, and maximum population density. The calculated 48 average values for GOL time and EGR are shown in Table 1. The maximum population density ranged between 10^6 and 10^8 CFU/ml in experiments where growth occurred, with most experiments showing a maximum population between 10^7 and 10^8 CFU/ml (data not shown).

B. stearothermophilus did not grow at pH 5.5 and 45°C at any level of NaCl concentration (Table 1) in the SCM. It also did not grow at 45°C and pH 6.0 with either 0 or 1% NaCl or at 45°C and pH 6.5 with 0% NaCl. GOL time was reduced by conditions favoring growth. The highest growth rate (1.02 h^{-1}) occurred at pH 7 and 55°C with 0.5% NaCl. The lowest measurable growth rate (0.14 h^{-1}) occurred at pH 5.5 and 50°C with 1.0% NaCl. The GOL time under these conditions was 28.9 h. The calculated GOL time and EGR values for the combination of NaCl and pH also indicated that decreasing temperature or decreasing pH increased GOL time and decreased EGR.

NaCl had the least effect on EGR and GOL time. It was expected that increasing the NaCl concentration from 0.5 to 1.0% would decrease EGR and increase GOL, and indeed the results in Table 1 support this expectation. The lower growth rates in 0% NaCl SCM versus those in 0.5% NaCl SCM were somewhat unexpected and will be discussed below.

The GOL time increased when the pH was decreased to 5.5, particularly at 50°C. *B. stearothermophilus* showed either no growth or extended GOL time at low pH values and low temperatures.

Comparison test for variables. The replications were not significantly different (results not shown), which means that the preexperimental storage time of spores and other day-to-day differences did not affect the GOL time and EGR ($P > 0.05$). All variables (pH, temperature, and NaCl concentration) affected the EGR and GOL time significantly ($P < 0.0001$).

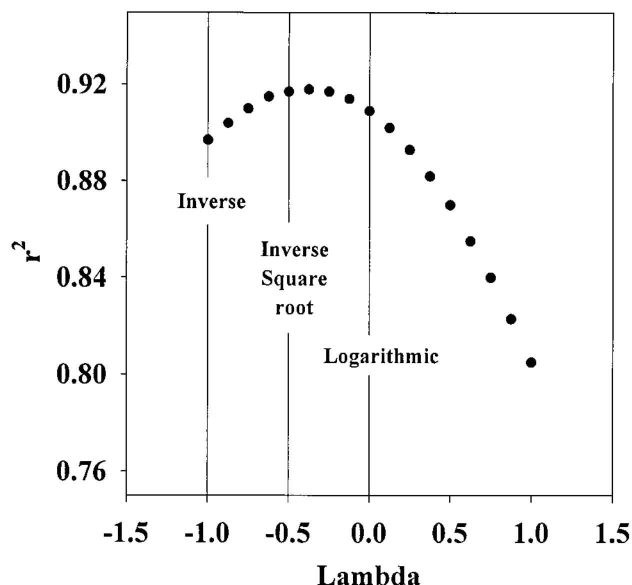

FIG. 1. The influence of the transformation (lambda) on the square of the correlation coefficient (r^2) for the response surface model for the effects of NaCl concentration, pH, and temperature on the GOL time of *B. stearothermophilus* in SCM. Standard transformations are indicated by vertical lines.

Therefore, these variables were considered to be important to the model.

The least significant difference test results (not shown) indicated that the relationship between GOL time or EGR and temperature, pH, or NaCl concentration was not linear; therefore, quadratic polynomial equations were used for the EGR and GOL time models.

Transformation and quadratic polynomial equations. The relationship between lambda and r^2 for response surface models of GOL time and EGR (equations 2 and 3, respectively) are shown in Fig. 1 and 2. Values for lambda are maximal at -0.375 and 0.75. This means that if GOL time and EGR data are raised to the λth power, the values of r^2 for the response surface models are the highest possible. It has been recommended, however, that use of the lambda transformation be avoided, because those nonstandard transformations are not easy to interpret (26). A useful alternative is to select the standard transformation (Fig. 1 and 2) that is closest to the lambda transformation and use that in the model. Based on this reasoning, the inverse square root transformation was used in the quadratic polynomial equation for GOL time. The closest standard transformation for the EGR data was no transformation at all.

The result of the response surface (quadratic polynomial) equations for the effects of NaCl, pH, and temperature on GOL time and EGR are presented in equations 2 and 3.

$$\begin{aligned}\mathrm{GOL}^{-0.5} = &-6.035 + 0.604(\mathrm{pH}) + 0.138(\mathrm{temperature}) + 0.599(\mathrm{NaCl}) \\ &-0.047(\mathrm{pH}^2) - 0.001(\mathrm{temperature}^2) - 0.311(\mathrm{NaCl}^2) \\ &-0.038(\mathrm{NaCl}\cdot\mathrm{pH}) - 0.0008(\mathrm{NaCl}\cdot\mathrm{temperature}) + 0.003(\mathrm{pH}\cdot\mathrm{temperature})\end{aligned} \quad (2)$$

$$\begin{aligned}\mathrm{EGR} = &-7.22 - 0.018(\mathrm{pH}) + 0.236(\mathrm{temperature}) + 0.291(\mathrm{NaCl}) \\ &-0.024(\mathrm{pH}^2) - 0.003(\mathrm{temperature}^2) - 0.736(\mathrm{NaCl}^2) \\ &+0.015(\mathrm{NaCl}\cdot\mathrm{pH}) + 0.009(\mathrm{NaCl}\cdot\mathrm{temperature}) + 0.01(\mathrm{pH}\cdot\mathrm{temperature})\end{aligned} \quad (3)$$

Both models have 10 parameters that include the intercept, the three variables, and their interactions. r^2 was used to indicate the fit of the models in the quadratic polynomial equations. The quadratic polynomial models of GOL time and EGR both showed high r^2 values, 0.917 (Fig. 1) and 0.916 (Fig. 2), respectively, which indicates that they fit the data well.

Quadratic versus cubic polynomial equation. Some reports in the literature used the cubic polynomial equation as well as the quadratic polynomial equation to fit predictive food microbiology data sets. Hudson (19) reported that cubic models gave significantly better fits than quadratic models, although negative values for lag time were sometimes predicted. Others have shown that quadratic models yield more realistic (i.e., positive) and accurate estimates of growth rate and lag time (25).

Increasing the power from quadratic to cubic would decrease the degrees of freedom of error of the model, which reduces its predictive accuracy. Every time a variable is added to a model, the fit should improve. If polynomial terms up through the 18th power were used, the data could be fitted exactly (27). One goal in making models is to reduce a large number of values into a concise information packet. If too many parameters are used to fit the data, the compactness of the results will be sacrificed, and the risk of random fluctuations producing accidental and erroneous effects is increased. Gauch (14) showed that the variance of noisy data around the values of a cubic equation was two times higher than the variance around a quadratic equation. This means that quadratic equations have good statistical efficiency and may actually be more accurate than the data used to generate the model.

Simplified model. Research papers on modeling have made use of response surface polynomial (RSP) regression (16, 19, 24, 25) and BS regression (9, 29) as tools to generate and measure the fit of predictive models. Stepwise regression eliminates variables or interactions which do not significantly contribute to the model. RSP regression, by contrast, takes into account all possible variables and their interactions in the

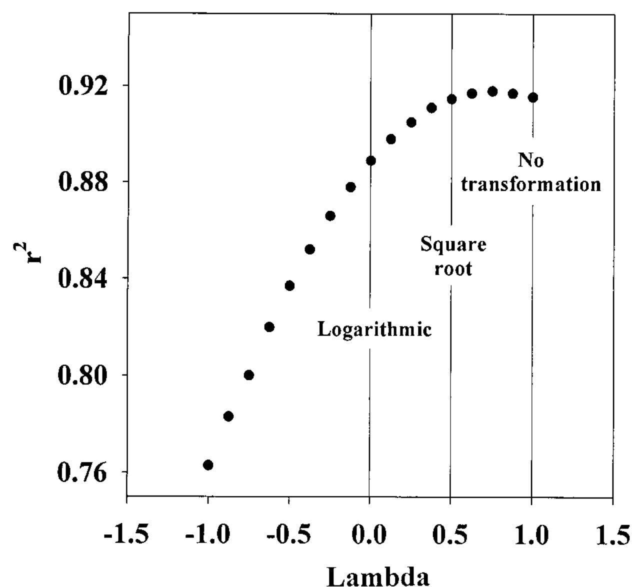

FIG. 2. The influence of the transformation (lambda) on the square of the correlation coefficient (r^2) for the response surface model for the effects of NaCl concentration, pH, and temperature on the EGR of *B. stearothermophilus* in SCM. Standard transformations are indicated by vertical lines.

TABLE 2. Squares of correlation coefficients (r^2) of modified quadratic polynomial models with BS regression

Modeled value	Regression procedure	Square of the correlation coefficient (r^2)	No. of parameters in the final model
$GOL^{-0.5}$	RSP	0.917	9
	BS	0.907	6
EGR	RSP	0.916	9
	BS	0.907	5

model. The r^2 produced by RSP regression will always be higher than that produced by stepwise regression. The disadvantage of RSP regression is that it may include terms in the model which are not statistically significant.

BS regression was used to eliminate insignificant variables, since these variables or their interactions did not provide any additional useful information to the model. The r^2 values for the equations simplified with BS regression are shown in Table 2. Three interaction terms were eliminated from the GOL time quadratic polynomial equation, while the r^2 was reduced insignificantly by 0.01. Four parameters were eliminated from the EGR quadratic polynomial equation, while the r^2 was reduced insignificantly by 0.009. The equations produced by BS regression are shown as equations 4 and 5.

$$GOL^{-0.5} = -7.415 + 0.806(\text{pH}) + 0.168(\text{temperature}) + 0.326(\text{NaCl}) - 0.052(\text{pH}^2) - 0.001(\text{temperature}^2) - 0.315(\text{NaCl}^2) \quad (4)$$

$$EGR = -8.093 + 0.269(\text{temperature}) - 0.003(\text{temperature}^2) - 0.689(\text{NaCl}^2) + 0.005(\text{pH} \cdot \text{temperature}) + 0.015(\text{NaCl} \cdot \text{temperature}) \quad (5)$$

The F values for model parameters can be used to indicate the significance of the effect of the variables and their interactions. BS regression parameters tend to have higher F values than RSP parameters in both modified equations (Table 3), because the elimination of insignificant parameters means that the remaining parameters take on a greater significance in determining the model prediction. The BS regression results show (Table 3) that each parameter remaining in the model is highly significant ($P < 0.0001$ in most cases).

The suitability of the BS versus the RSP model for describing the data can be determined with a lack-of-fit test, where the null hypothesis is that the simplified models represent the data. The alternative hypothesis is that the quadratic models represent the data. An F statistic for lack of fit (F_{LOF}) is calculated, based on the residual sum of squared errors. If F_{LOF} falls in the range between zero and a tabular F value (based on a particular α [0.05 in this case] and the degrees of freedom of the simple and full models), the simplified model is selected. The results of the F_{LOF} values for both EGR and GOL time models were such that the simplified quadratic polynomial equations were selected (results not shown).

Explanation of parameters and interactions. The parameters from equations 4 and 5 and their statistical significance (Table 3) can be used to gain some insight into possible mechanisms influencing *B. stearothermophilus* growth and their suitability and importance for controlling the growth of this food spoilage microbe.

Considering GOL time first, of the simple terms remaining in equation 4, Table 3 indicates that NaCl and temperature are the most significant (higher F values). This means that changes in these environmental factors will have a very significant effect on *B. stearothermophilus* GOL time. The squares of these two terms also have highly significant F values. The presence of these squared terms in equation 4 means that when NaCl concentration and temperature change, their effect on GOL is nonlinear, i.e., an increase from 45 to 50°C will produce a greater change in GOL than an increase from 55 to 60°C. This nonlinearity is compounded for equation 4 because of the transformation ($GOL^{-0.5}$) used. This transformation indicates a nonlinear response of GOL time to all of the variables, and the presence of squared terms in the model makes this nonlinearity even greater.

TABLE 3. F values for significant variables and their interactions for quadratic polynomial models of EGR after RSP or BS regression

Modeled value and variable	F value	
	RSP	BS
$GOL^{-0.5}$		
Intercept	13.68[b]	42.45[a]
NaCl	5.57	41.73[a]
pH	3.31	7.86[b]
Temperature	15.59[b]	42.34[a]
$NaCl^2$	42.58[a]	43.25[a]
pH^2	4.18	5.16[b]
$Temperature^2$	27.73[a]	35.95[a]
NaCl · temperature	0.07	
NaCl · pH	2.18	
pH · temperature	1.68	
EGR		
Intercept	6.76[b]	23.56[a]
NaCl	0.38	
pH	0.00	
Temperature	15.82[b]	25.38[a]
$NaCl^2$	46.35[a]	46.44[a]
pH^2	0.22	
$Temperature^2$	25.84[a]	25.91[a]
NaCl · temperature	2.34	55.47[a]
NaCl · pH	0.07	
pH · temperature	6.55[b]	100.83[a]

[a] $P > F$ value $= 0.0001$.
[b] $P > F$ value $= 0.05$.

Most simple chemical and biochemical reactions show a dependence on temperature governed by Arrhenius rate kinetics (33):

$$k = Ae^{-\frac{E_a}{RT}} \quad (6)$$

where k is the reaction rate, A and R are constants, E_a is the reaction activation energy, and T is the absolute temperature. These kinetics don't describe multienzyme microbial growth very well. A complex version of the simple Arrhenius equation which appears to describe the effect of temperature on microbial growth fairly well has been developed (1, 28). This model supposes two or three key enzymes, each controlling growth in a specific temperature range for a given microbe. Because of the nonlinearity indicated by our models, this suggests that these more complex Arrhenius-style kinetics may be applicable here and that the effect of temperature on *B. stearothermophilus* growth may be through several key and as-yet-unidentified enzymes.

The large size of the $NaCl^2$ term in equation 4 (−0.315) which approaches the size of the linear NaCl term (0.326) indicates an optimum NaCl concentration for the shortest GOL time. As mentioned above, the data indicate an optimum around 0.5% NaCl. It is known that NaCl generally has an inhibitory effect on microbial growth. *Bacillus* spore recovery after injury, for example, is slowed by an increased NaCl level (6, 10) when its concentration is too high. Increasing levels of NaCl have also been shown to increase lag time and lower the growth rate of *Bacillus cereus* (3). The lower growth rate in 0% NaCl SCM versus that in 0.5% NaCl SCM was somewhat unexpected, however. The lowest concentration of NaCl in many other modeling experiments (8, 24, 31) was 0.5%, because this is the typical NaCl content in many microbial growth media. This study used a medium (SCM) where NaCl concentration could be controlled at any level, permitting the use of NaCl concentrations less than 0.5%. These results seem to indicate an optimum NaCl level for *B. stearothermophilus* growth. It is known that *Escherichia coli* cells maintain a constant Na^+ ion concentration even when medium osmolality is varied (20), indicating its importance in a variety of cellular processes. This same importance may also be true for *Bacillus* species. If extracellular concentrations vary too widely, the ultimate impact may be to slow growth or lengthen GOL time. A recent study has reported a salt-dependent alpha-amylase gene from *Bacillus circulans* (22). If a similar gene exists in *B. stearothermophilus*, this could also explain our findings. At low salt concentrations, this gene would be turned off and thus would slow growth because of slower starch hydrolysis, and subsequently less glucose would be available to support growth.

The pH effect on GOL time, while measurable ($P < 0.05$), is not as statistically significant as the effects of NaCl concentration and temperature ($P < 0.0001$). This lower significance may imply greater experimental variability in setting the medium pH. It may also imply that if more data were collected, a model with more complex pH interactions would emerge. Table 3 does show an interaction between NaCl concentration and pH ($F = 2.18$) in the full model which was not significant enough to be part of the final model. Despite this low statistical significance, the high value for the pH parameter in equation 4 means that pH value does have great practical significance. Lowering a food's pH would give appreciable extension of shelf life with respect to *B. stearothermophilus* spoilage.

The effect of pH on microbial growth is known to show an optimum, usually around 7, and a minimum and maximum as well (21). The nonlinearity implied by equation 4 supports this basic fact for *B. stearothermophilus* in the system studied here. Enzymes are known to have pH optima similar to those shown by whole cells. pH value is also known to affect transport of nutrients into the cell. Our models suggest that either of these two mechanisms may be operating here.

Many of the comments concerning the GOL time model (equation 4) are also applicable to the EGR model (equation 5). The EGR model shows a quadratic temperature effect because of significant temperature and $temperature^2$ terms (Table 3). This model also shows a quadratic NaCl effect because of its $NaCl^2$ term (Table 3). The EGR model also has several noticeable differences versus the GOL time model, because it contains two very significant interaction terms, NaCl · temperature and pH · temperature ($F = 55.47$ and 100.83, respectively).

Extreme care should be used in interpretation of the statistical and practical significance of interaction effects, with an emphasis on avoiding overinterpretation, especially where the simple linear terms do not appear in the final model. Considering the pH · temperature effect: if the regression analysis is rerun, with instructions not to include this interaction term, a pH term appears in the final model, with high ($F = 96.57$) significance (results not shown), while the rest of the model is largely unchanged, and the r^2 is reduced only slightly (0.899). In this case, this means that while a pH · temperature term is appropriate, changing the model to include a simple pH term instead may be almost as suitable and may be much easier to interpret biologically. Medium pH is known to affect microbial behavior as discussed above, and it may be, for the system under consideration, that in the limited pH range from 5.5 to 7.0 its effect on the growth rate is linear. It is also possible that a true interaction between temperature and pH does exist. This would indicate a synergistic effect on the structure of a key enzyme or nutrient transport pathway.

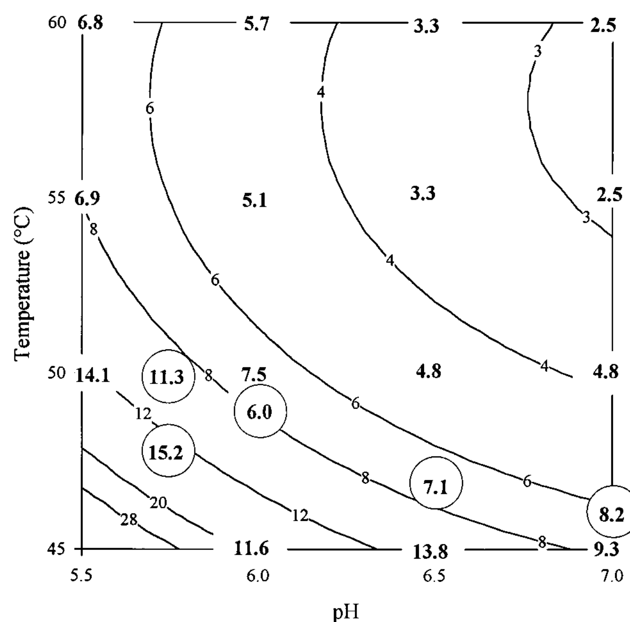

FIG. 3. Validation of a mathematical model for the effects of temperature and pH on the GOL time of *B. stearothermophilus* at 0.5% NaCl. Contour lines represent model predictions. Boldfaced numbers are experimental data used to generate the model. Numbers within circles are validation results not used to generate the model predictions.

Similar care should be taken in the interpretation of the NaCl · temperature interaction. If this regression analysis is rerun, with instructions not to include the NaCl · temperature interaction term, an NaCl term appears in the final model, with high ($F = 53.45$) significance (results not shown), while the rest of the model is largely unchanged, and the r^2 is reduced only slightly (0.906). This means that while these data support an interaction as the best way to describe the *B. stearothermophilus* growth rate in this system, less biologically complex explanations may also be suitable. If the interaction effect is real, however, this points to a possible temperature dependence of the putative salt-dependent α-amylase mentioned above.

Model validation. The validation results are shown in Fig. 3 and 4 and indicate a high degree of correlation for both the GOL time and EGR models. The figures show GOL time or EGR model contour lines between 45 and 60°C and pH 5.5 and pH 7.0 for 0.5% salt concentration. The actual experimental data and validated data are also shown (see the legends to Fig. 3 and 4).

The predicted GOL times and EGRs can be determined for

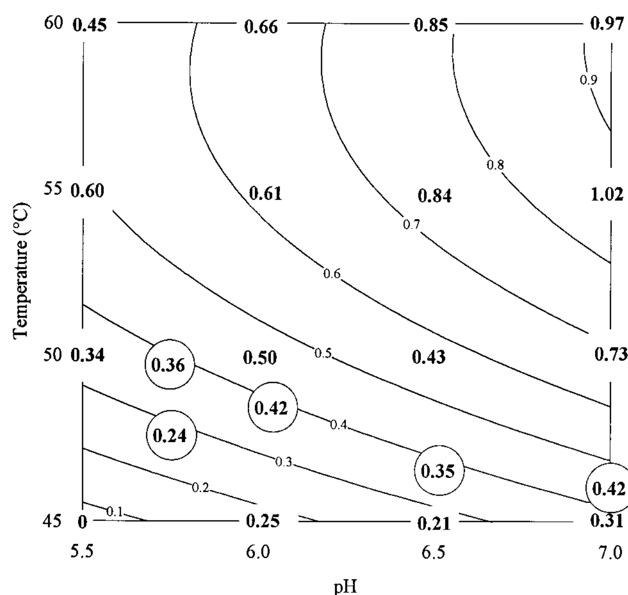

FIG. 4. Validation of a mathematical model for the effects of temperature and pH on the EGR of *B. stearothermophilus* at 0.5% NaCl. Contour lines represent model predictions. Boldfaced numbers are experimental data used to generate the model. Numbers within circles are validation results not used to generate the model predictions.

any SCM formulation with equations 4 and 5 or the contour plots. The GOL time and EGR models can be used to develop new food products, reformulate existing food products, or predict shelf life for currently produced thermostabilized foods. These models will be useful for food processors who must manufacture for both the civilian and military markets, where high temperature stability is needed. Further research is currently under way to validate these models in real food systems.

Conclusion. A first approach to model the effects of NaCl concentration, pH, and temperature on the kinetics of *B. stearothermophilus* in thermostabilized foods has been presented. The results of this study can be applied in the formulation of foods that rely on the variables investigated to inhibit the growth of *B. stearothermophilus*. Food product formulations may use the different variable levels that showed growth inhibition of *B. stearothermophilus* to improve the quality and extend the shelf life of thermostabilized food products stored at elevated temperatures.

Furthermore, these models can also be applied to existing thermostabilized food systems. If the pH and NaCl concentration are known, these models will determine the appropriate storage temperature required to ensure adequate microbiological shelf life.

ACKNOWLEDGMENTS

Thanks go to Frank Busta for suggesting the term GOL time. Thanks also go to the two anonymous reviewers for their helpful critique of the manuscript, especially for their suggestion to discuss the interaction effects more extensively.

This work was supported by funds from the state of New Jersey and by the Center for Advanced Food Technology (CAFT). CAFT is a New Jersey Commission on Science and Technology Center. This work was supported in part by the U.S. Army Research Office.

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