A Radial Basis Function Neural Network Approach To Determine the Survival of *Listeria monocytogenes* in Katiki, a Traditional Greek Soft Cheese

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**ABSTRACT**

A radial basis function neural network was developed to determine the kinetic behavior of *Listeria monocytogenes* in Katiki, a traditional white acid-curd soft spreadable cheese. The applicability of the neural network approach was compared with the reparameterized Gompertz, the modified Weibull, and the Geeraerd primary models. Model performance was assessed with the root mean square error of the residuals of the model (RMSE), the regression coefficient ($R^2$), and the $F$ test. Commercially prepared cheese samples were artificially inoculated with a five-strain cocktail of *L. monocytogenes*, with an initial concentration of $10^6$ CFU g$^{-1}$ and stored at 5, 10, 15, and 20°C for 40 days. At each storage temperature, a pathogen viability loss profile was evident and included a shoulder, a log-linear phase, and a tailing phase. The developed neural network described the survival of *L. monocytogenes* equally well or slightly better than did the three primary models. The performance indices for the training subset of the network were $R^2 = 0.993$ and RMSE = 0.214. The relevant mean values for all storage temperatures were $R^2 = 0.981, 0.986$, and 0.985 and RMSE = 0.344, 0.256, and 0.262 for the reparameterized Gompertz, modified Weibull, and Geeraerd models, respectively. The results of the $F$ test indicated that none of the primary models were able to describe accurately the survival of the pathogen at 5°C, whereas with the neural network all $f$ values were significant. The neural network and primary models all were validated under constant temperature storage conditions (12 and 17°C). First or second order polynomial models were used to relate the inactivation parameters to temperature, whereas the neural network was used a one-step modeling approach. Comparison of the prediction capability was based on bias and accuracy factors and on the goodness-of-fit index. The prediction performance of the neural network approach was equal to that of the primary models at both validation temperatures. The results of this work could increase the knowledge basis for the applicability of neural networks as an alternative tool in predictive microbiology.

Katiki is a creamy white acid-curd soft cheese with a mild sour and salty taste. It is made from goats’ milk or a combination of goats’ and ewes’ milk, which is popular in Greek and other Mediterranean markets. Katiki is a traditional PDO (protected designation of origin) soft cheese produced primarily in the area of Domokos in central Greece. The product is one of the unripened soft cheeses, which are consumed fresh within a few days after manufacture. According to the traditional cheese-making practices employed by most small dairies in the area, the raw milk is pasteurized, cooled to ca. 25°C, and then inoculated with a mesophilic starter culture of lactic acid bacteria to facilitate coagulation and acidification. Occasionally, a small amount of rennet may be added when the pH reaches 6.0 to 6.2. After 24 h, the curd is transferred to clean cheese cloth for draining. When the moisture of the cheese is ca. 72%, salt is mixed in to produce a homogeneous cheese mass, which is packaged and stored at 4°C. The final characteristics of the product are 75% moisture, 40% fat in dry matter, 8.8% protein, 1.5 to 2% salt, and pH of 4.3 to 4.5 (74).

*Listeria monocytogenes* is a gram-positive nonsporulated facultatively anaerobic bacterium that is widely distributed in the natural environment under diverse conditions; it can grow at refrigeration temperatures and survive in foods for prolonged periods under adverse conditions (41). Growth and/or survival can occur over a wide range of pH values (4.3 to 9.1) and temperatures (−1.5 to 45°C) (18, 35, 38), high salt concentrations (10 to 14%), and low water activity ($a_w$) levels (17, 49, 54). The pathogen is of great public health concern because of the high mortality rate associated with infection in specific target groups, such as pregnant women, newborns, the elderly, and the immunocompromised (1, 50, 60). Generally, the heat treatment applied to raw milk during cheese manufacture is sufficient to inactivate small populations of the *L. monocytogenes* that may be present but does not eliminate later risk of postprocessing contamination in dairy facilities (44, 72). The combination of artisan practices employed in Katiki manufacture and the potential for poor hygienic conditions in certain small dairies increased the risk for *L. monocytogenes* cross-contamination and survival in this cheese throughout the retail chain through the time of consumption. Although there are no data available about the prevalence of *L. monocytogenes* in Katiki and other Greek soft cheeses, in several surveys undertaken in Italy the pathogen was recovered in

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about 3% of Italian cheeses, with a prevalence of 7% in gorgonzola, 6% in mozzarella, and 4% in various other soft cheeses (64). The presence of *L. monocytogenes* has been reported, in lower or higher numbers, in other studies performed to evaluate microbial contamination in Italian soft cheeses (24, 45, 57, 67).

Predictive microbiology recently has been employed extensively as a valuable tool to describe quantitatively the kinetic behavior of pathogenic and spoilage microorganisms under certain environmental conditions, providing a rapid and cost-effective method for the quantitative assessment of microbial growth for product development, risk assessment, and educational purposes (63). Predictive microbiology is based on a two-step approach of primary and secondary modeling. The models that describe the response of a microorganism to a single set of conditions over time are termed primary models, and those that describe the effect of environmental factors on the values of the parameters of the primary models are the secondary models. Response surface models are frequently used to describe the relationships between environmental factors (e.g., pH, aw, temperature, and preservatives) and bacterial kinetic parameters (6, 9, 10, 48, 71), but new methodologies are being introduced, such as the application of neural networks (NNs) (5, 34, 37). Interest in using NNs as modeling tools in food science and technology is increasing; these models have been successfully employed in several food applications such as sensory analysis, pattern recognition, classification, microbial predictions, and food process optimization (2, 22, 27, 40, 53, 66). NNs are computing algorithms designed to imitate the computational capabilities of large, highly connected networks of relatively simple elements such as neurons in the human brain (4). These algorithms contain a series of mathematical equations that are used to simulate biological processes such as learning and memory.

NN development involves a learning process that adaptively responds to the input variables according to a learning rule. The network has the ability to learn from its environment and adapt to it, as do its biological counterparts (30). An NN normally has no restriction on the type of relationship between the growth parameters (input patterns) and the desired output. In contrast to conventional models in which a mathematical equation must be stated beforehand, NNs directly explore the knowledge contained in the input-output patterns by adjusting the highly nonlinear NN topology as the input-output patterns are repeatedly presented to the network (28). In operation, when the network is trained on the appropriate data set (supervised learning), it can then be used to predict values for different growth conditions within the initial experimental range. Compared with conventional modeling, an NN could be used as a one-step approach providing fitting of a microorganism under a given set of conditions while predicting the response-time curves under a different set of conditions.

The aim of this research was (i) to investigate the use of NNs to determine the survival of inoculated *L. monocytogenes* in Katiki during storage at constant temperatures (5, 10, 15, and 20°C), (ii) to compare the performance of NNs with those of statistical models developed on the same data set, and (iii) to validate the prediction capability (generalization) of the NN approach at two other intermediate temperatures (12 and 17°C) not initially employed in the development of the network.

**MATERIALS AND METHODS**

**Bacteria and preparation of inocula.** Five strains of *L. monocytogenes* were used throughout this study: NCTC 10527, Scott A, LMBF-123, LMBF-131, and LMBF-133 (LMBF isolates were from our collection and the serotypes were not known). All strains except NCTC 10527 were previously isolated from cheese samples. Stock cultures were maintained in vials of treated beads in a cryoprotective fluid (Protect Bacterial Preservers, Technical Service Consultants Ltd., Heywood, UK) at −80°C until use. The cultures were revived by inoculation in 9 ml of tryptic soy broth (TSB; 402155, Biolife, Milan, Italy) and incubation at 30°C for 24 h. For experiments, a loopful of each strain was transferred into 9 ml of TSB and subcultured twice at 30°C for 24 h.

**Cheese inoculation and sampling.** Packages (180 g) of commercially prepared Katiki, obtained from a local super market, were inoculated with a five-strain cocktail of *L. monocytogenes*. The cocktail was prepared by combining individual cultures in sterile tubes and centrifuging at 6,000 rpm for 30 min at 4°C. The resulting pellet was washed with sterile Ringer’s solution, recentrifuged, and resuspended in the same diluent to a final volume of 4.5 ml. The cell concentration in the resulting composite inoculum was 9.0 log CFU ml⁻¹, as assessed with a Neubauer counting chamber (Brand, Wertheim, Germany), and this inoculum was used for all experiments. The volume of the inoculum added to each package was 180 μl to give an initial population density of ca. 10⁶ CFU g⁻¹ as determined in preliminary trials. To ensure uniform distribution of the inoculum, the cheese was thoroughly stirred with a sterile spatula. One hundred twenty packages were inoculated (i.e., 30 for each temperature) and stored at 5, 10, 15, and 20°C in high precision (±0.5°C) incubation chambers (MIR-153, Sanyo Electric Co., Osaka, Japan). Uninoculated packages also were held under the same conditions and served as controls. The experiment was repeated twice with duplicate cheese packages for each sampling point (n = 4).

**Microbiological analyses.** At regular intervals, depending on each incubation temperature, 25-g cheese samples were transferred aseptically into a stomacher bag, and 225 ml of 0.25% sterile Ringer’s solution was added. The mixture was homogenized for 60 s at room temperature (ca. 20°C) in a stomacher (Lab-Blender 400, Seward Medical, London, UK). Further decimal dilutions were prepared with the same diluent and duplicate 1- or 0.1-ml samples of three appropriate dilutions were mixed or spread in triplicate on total count and selective agars (Biolife): *Listeria* PALCAM agar base for enumeration of listeriae was incubated at 30°C for 48 h; deMan Rogosa Sharpe medium adjusted to pH 5.7 for lactic acid bacteria was overlaid with the same medium and incubated at 30°C for 72 h; and plate count agar for total counts of viable bacteria was incubated at 30°C for 72 h. Growth data from plate counts were log transformed, a well-known technique used with microbiological data to stabilize the variance (36). Cheese samples were routinely analyzed for pH with a pH meter (model RL 150, Russell Inc., Boston, Mass.).

**Model development.** Different primary models were fitted to the data set to determine the kinetic parameters of *L. monocytogenes* in soft cheese at different storage temperatures. The first model applied was the reparameterized Gompertz model (76) determined by the following equation:
\[
\log N(t) = \log N(0) + A \exp \left( -\frac{k \cdot e}{A} (t_s - t) + 1 \right)
\]

where \( t_s \) is the duration of the shoulder (days), \( k \) is the maximum specific inactivation rate per day, \( N(0) \) is the initial population density (log CFU per gram), and \( A \) is the difference between the initial and residual population (log CFU per gram).

The second modeling approach was based on the modified Weibull model (3), which can be written as follows:

\[
\log N(t) = \log ([(N(0) - N_{\text{res}}) \cdot 10^{-(\delta \cdot p)} + N_{\text{res}}])
\]

where \( \delta \) is a scale parameter that denotes the time (days) for the first decimal reduction and \( p \) is the shape factor of the curve. For \( p > 1 \), convex curves are obtained, whereas for \( p < 1 \) concave curves are described. \( N(0) \) and \( N_{\text{res}} \) are the initial and residual populations (log CFU per gram), respectively.

The Geeraerd model (23) was the third modeling approach employed, defined by the following equation:

\[
\log N(t) = \log \left[ \frac{N(0) - N_{\text{res}} \cdot e^{-k_{\text{max}}t}}{1 + (e^{k_{\text{max}}t} - 1) e^{-k_{\text{max}}t}} + N_{\text{res}} \right]
\]

where \( k_{\text{max}} \) is the maximum specific inactivation rate per day, \( t_s \) is the duration of the shoulder (days), and \( N(0) \) and \( N_{\text{res}} \) are the initial and residual populations (log CFU per gram), respectively.

**Artificial NNs.** In this study, an NN approach was employed as a one-step model to determine its fit to the survival pattern of *L. monocytogenes*. The NN type chosen was a radial basis function (RBF), which consists of three layers: an input layer, a single hidden layer where the nodes are Gaussian kernels, and a linear output layer. The activation of a neuron in the hidden layer is that represents the center of the basis functions. The spread \( (w_i) \) in the linear layer of the network are determined by applying the orthogonal least-squares (OLS) algorithm originating from linear regression models. The mapping performed by the network can be viewed as a regression model of the form

\[
Y_d = \Phi \cdot W + E
\]

where \( Y_d \) is the vector of desired network outputs, \( \Phi \) is a regression vector, \( W \) is the vector of weights, and \( E \) is the vector of errors between the desired and actual network outputs. The centers of the Gaussian kernels and the values of the weights \( (w_i) \) in the linear layer of the network are determined by clustering techniques or random selections. Another method for optimizing the parameters of the RBF is the gradient descent algorithm (39).

The spreads \( (\sigma) \) of the basis functions are computed based on the heuristic rule “global first nearest neighbor” (51). The width of the radial basis \( i \) centered at \( c_i \) is set to the Euclidean distance between \( c_i \) and its nearest neighbor center or candidate center \( c_j \) multiplied by an overlap constant \( q \), such that

\[
\sigma_i = \frac{q}{\sqrt{-2 \log 0.5}} \min(||c_i - c_j||)
\]

The centers of the Gaussian kernels and the values of the weights \( (w_i) \) in the linear layer of the network are determined by applying the orthogonal least-squares (OLS) algorithm originating from linear regression models. The mapping performed by the network can be viewed as a regression model of the form

\[
Y_d = \Phi \cdot W + E
\]

where \( Y_d \) is the vector of desired network outputs, \( \Phi \) is a regression vector, \( W \) is the vector of weights, and \( E \) is the vector of errors between the desired and actual network outputs. The centers of the network are chosen from the set of input patterns. This article does not provide an in-depth analysis of the mathematical basis of RBF NN development; more details about the application of this kind of networks in predictive microbiology can be found elsewhere (55).

In equation 4, \( x \) corresponds to input information (independent variables) introduced in the network. In our case, \( x \) refers to two input vectors: storage temperature and time. The desired output (dependent variable) is the microbiological counts (log CFU per gram) of *L. monocytogenes* at the selected time interval. The entire database consisted of 71 experimental inactivation data points (i.e., storage temperatures \( \times \) sampling points). A standard procedure was followed for NN development by dividing the database into training and test subsets (29). The training subset consisted of the survival data of *L. monocytogenes* at 5, 10, 15, and 20°C (45 data points), whereas the generalization capability (prediction) of the network was tested on the remaining 26 data points at 12 and 17°C.

The algorithm of the RBF network was developed in MATLAB version 7.0 code (Mathworks, Inc., Natick, Mass.), whereas the reparameterized Gompertz, modified Weibull, and Geeraerd models were fitted by the nonlinear regression procedure in Statistica software version 6.0 (Statsoft, Inc., Tulsa, Okla.).

**Performance evaluation.** The following three statistical indices were used to compare the models: the root mean squared error (RMSE) of the residuals of the model, the regression coefficient \( (R^2) \), and the F test statistic, as defined elsewhere (15, 47, 68).

**Model validation.** The NN and statistical models were evaluated to determine whether they could predict the survival of *L.
**RESULTS AND DISCUSSION**

The population dynamics of the five-strain cocktail of *L. monocytogenes* in Katiki during storage at 5, 10, 15, and 20°C is presented in Figure 1. The pathogen was not detected in any of the control cheeses (commercial noninoculated packages) stored under the same conditions. At each storage temperature assayed, a clear inactivation pattern was evident comprising a shoulder, a log-linear phase, and a tailing phase. The estimated kinetic parameters and statistical indices of the survival curves fitted with the reparameterized Gompertz, the modified Weibull, and the Geeraerd models are shown in Table 1. All models fitted the survival of *L. monocytogenes* accurately, as can be inferred from the performance of the relevant indices ($R^2 > 0.96$; RMSE $< 0.371$). However, the $F$ test revealed that all models were inadequate to describe the data at 5°C, as the $f$ value was not significant at $P < 0.05$. The RBF NN also fitted the data well, as indicated by the lower RMSE and higher $R^2$ values compared with those in the statistical models. The NN approach also was able to describe the inactivation data well at 5°C, as indicated by the significant $f$ value. The developed NN, designated NN$_{2,m-1}$, consisted of two input nodes of storage temperature ($T$) and sampling time ($t$), an output node for the surviving population of *L. monocytogenes* (log CFU per gram), and a hidden layer with $m$ Gaussian kernels, each with a fixed spread $\sigma$ ($m$ and $\sigma$ to be determined). Using the “global first nearest neighbor” rule (equation 6), the common spread for all Gaussian kernels was calculated as 12. The corresponding centers ($c_i$) were determined by the OLS procedure (equation 7) as 15. The values of the output connection weights ($w_i$) in the linear layer of the network and the associate centers ($c_i$) of the Gaussian kernels are presented in Table 2.

The decrease in the population of *L. monocytogenes* could be attributed to the inhibitory effect of low pH and...
the activity of lactic acid bacteria starter cultures added during cheese manufacture. The growth of lactic acid bacteria followed the growth of total viable bacteria and increased steadily from 6.5 to 6.2 log CFU g\(^{-1}\) to 8.2 to 8.0 log CFU g\(^{-1}\), whereas a slight decrease from pH 4.6 to 4.5 to pH 4.3 to 4.2 was observed (data not shown). The pH values in cheese were close to the tolerance limit for growth of L. monocytogenes (44, 58), and inhibition of the pathogen by low pH has been reported previously for other soft cheeses (33, 46, 52, 56, 59, 61, 65). The presence of tailing in all survival curves could be attributed to heterogeneity in bacterial cells and to the development of defensive mechanisms in response to acid stress, such as modification of fatty acid composition of the membrane and development of the acid tolerance response (7, 12).

The RBF NN also was used to predict the survival of L. monocytogenes at two intermediate storage temperatures, 12 and 17°C. Generally, to model time-response curves, two procedures are available: a one-step procedure and a two-step procedure. In the first approach, all curves are fitted at the same time by a one-step model that employs time and all the environmental factors as independent variables (73). In this work, the RBF NN was used as a one-step model to investigate the capability to simultaneously fit and predict the response-time survival curves of L. monocytogenes in Katiki. Following a standard procedure in NN methodology, the whole data set was divided into (i) a training subset containing the survival data of the pathogen at 5, 10, 15, and 20°C, which was utilized for network development (i.e., calculation of centers, spread, and weights), and (ii) a validation subset (survival data at 12 and 17°C), which was used to test the generalization (prediction) capability of the network. For this NN, the relevant performance indices were \(R^2\) (training) = 0.991, \(R^2\) (validation) = 0.84, and \(R^2\) (testing) = 0.79.

### TABLE 1. Parameter estimation and statistical indices of the different models used for fitting the experimental data\(^a\)

<table>
<thead>
<tr>
<th>Model type</th>
<th>(N_0) (log CFU g(^{-1}))</th>
<th>(A^b) (log CFU g(^{-1}))</th>
<th>(N_{res}) (log CFU g(^{-1}))</th>
<th>(k_{max}) (day(^{-1}))</th>
<th>(t_c) (days)</th>
<th>(\delta) (days)</th>
<th>(p)</th>
<th>RMSE</th>
<th>(R^2)</th>
<th>(f)</th>
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<tbody>
<tr>
<td>rGompertz(^c)</td>
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<tr>
<td>5°C</td>
<td>6.13 ± 0.24</td>
<td>4.21 ± 0.31</td>
<td>0.71 ± 0.14</td>
<td>4.96 ± 1.55</td>
<td>0.371</td>
<td>0.967</td>
<td>4.62(^d)</td>
<td>0.99</td>
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<tr>
<td>10°C</td>
<td>5.98 ± 0.24</td>
<td>4.35 ± 0.32</td>
<td>0.62 ± 0.11</td>
<td>3.87 ± 0.96</td>
<td>0.334</td>
<td>0.985</td>
<td>1.35</td>
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<tr>
<td>15°C</td>
<td>5.96 ± 0.12</td>
<td>4.34 ± 0.18</td>
<td>0.87 ± 0.09</td>
<td>3.46 ± 0.34</td>
<td>0.249</td>
<td>0.992</td>
<td>0.93</td>
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<tr>
<td>20°C</td>
<td>5.71 ± 0.21</td>
<td>4.38 ± 0.32</td>
<td>1.37 ± 0.32</td>
<td>2.85 ± 0.55</td>
<td>0.422</td>
<td>0.978</td>
<td>2.13</td>
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<tr>
<td>mWeibull(^e)</td>
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<tr>
<td>5°C</td>
<td>6.26 ± 0.25</td>
<td>1.96 ± 0.13</td>
<td>5.81 ± 1.22</td>
<td>1.88 ± 0.67</td>
<td>0.336</td>
<td>0.976</td>
<td>3.78(^d)</td>
<td>0.99</td>
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<tr>
<td>10°C</td>
<td>6.22 ± 0.23</td>
<td>1.71 ± 0.13</td>
<td>4.38 ± 0.84</td>
<td>1.44 ± 0.27</td>
<td>0.265</td>
<td>0.985</td>
<td>0.85</td>
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<tr>
<td>15°C</td>
<td>6.13 ± 0.13</td>
<td>1.71 ± 0.11</td>
<td>4.10 ± 0.37</td>
<td>1.93 ± 0.25</td>
<td>0.186</td>
<td>0.992</td>
<td>0.52</td>
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<tr>
<td>20°C</td>
<td>6.01 ± 0.25</td>
<td>1.45 ± 0.17</td>
<td>2.87 ± 0.47</td>
<td>1.94 ± 0.43</td>
<td>0.236</td>
<td>0.989</td>
<td>0.67</td>
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<tr>
<td>Geeraerd</td>
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<tr>
<td>5°C</td>
<td>6.26 ± 0.23</td>
<td>1.88 ± 0.13</td>
<td>0.81 ± 0.14</td>
<td>2.84 ± 1.41</td>
<td>0.301</td>
<td>0.981</td>
<td>3.05(^d)</td>
<td>0.99</td>
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<tr>
<td>10°C</td>
<td>6.11 ± 0.21</td>
<td>1.69 ± 0.13</td>
<td>1.01 ± 0.11</td>
<td>2.70 ± 0.92</td>
<td>0.245</td>
<td>0.987</td>
<td>0.73</td>
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<tr>
<td>15°C</td>
<td>6.02 ± 0.09</td>
<td>1.71 ± 0.08</td>
<td>1.61 ± 0.12</td>
<td>3.01 ± 0.30</td>
<td>0.145</td>
<td>0.995</td>
<td>0.32</td>
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<tr>
<td>20°C</td>
<td>5.87 ± 0.25</td>
<td>1.43 ± 0.20</td>
<td>2.38 ± 0.40</td>
<td>2.22 ± 0.54</td>
<td>0.357</td>
<td>0.976</td>
<td>1.52</td>
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<tr>
<td>RBF NN(^f)</td>
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<td>5°C</td>
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<td></td>
<td>0.218</td>
<td>0.992</td>
<td>1.59</td>
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<tr>
<td>10°C</td>
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<td>0.216</td>
<td>0.993</td>
<td>0.57</td>
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<tr>
<td>15°C</td>
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<td>0.155</td>
<td>0.996</td>
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<tr>
<td>20°C</td>
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<td></td>
<td>0.265</td>
<td>0.989</td>
<td>0.84</td>
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</tbody>
</table>

\(^a\) Data are mean ± standard error.

\(^b\) \(A\) is the difference between the initial population (\(N_0\)) and the residual population (\(N_{res}\)).

\(^c\) Reparameterized Gompertz.

\(^d\) Not significant (\(P < 0.05\)).

\(^e\) Modified Weibull.

\(^f\) Radial basis function neural network.

### TABLE 2. Connection weights (\(w_i\)) from the hidden layer (15 Gaussian nodes) to the output layer (1 linear unit) and calculated centers (\(c_i\)) of the RBF network using the OLS learning scheme

<table>
<thead>
<tr>
<th>Id node ((j))</th>
<th>Connection weights ((w_i))</th>
<th>Calculated centers ((c_i))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−0.0052</td>
<td>10, 0</td>
</tr>
<tr>
<td>2</td>
<td>−0.0025</td>
<td>5, 23</td>
</tr>
<tr>
<td>3</td>
<td>−0.9814</td>
<td>20, 0</td>
</tr>
<tr>
<td>4</td>
<td>0.0056</td>
<td>5, 1</td>
</tr>
<tr>
<td>5</td>
<td>2.0652</td>
<td>20, 1</td>
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<tr>
<td>6</td>
<td>−0.0006</td>
<td>5, 39</td>
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<tr>
<td>7</td>
<td>−0.0287</td>
<td>15, 8</td>
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<tr>
<td>8</td>
<td>0.5352</td>
<td>15, 0</td>
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<tr>
<td>9</td>
<td>−0.0104</td>
<td>15, 18</td>
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<tr>
<td>10</td>
<td>−1.1591</td>
<td>15, 1</td>
</tr>
<tr>
<td>11</td>
<td>0.6591</td>
<td>15, 2</td>
</tr>
<tr>
<td>12</td>
<td>0.0342</td>
<td>20, 10</td>
</tr>
<tr>
<td>13</td>
<td>−0.0032</td>
<td>5, 15</td>
</tr>
<tr>
<td>14</td>
<td>−1.1202</td>
<td>20, 2</td>
</tr>
<tr>
<td>15</td>
<td>0.0102</td>
<td>10, 18</td>
</tr>
</tbody>
</table>
TABLE 3. Parameters and statistics of secondary models for the effect of temperature on the kinetic parameters of Listeria monocytogenes in Katiki

<table>
<thead>
<tr>
<th>Model type</th>
<th>Parameter</th>
<th>Equation</th>
<th>Estimated value&lt;br&gt;&lt;br&gt; $^a$</th>
<th>$P$</th>
<th>$R^2$</th>
</tr>
</thead>
</table>
| rGompertz     | $k_{\max}$ | $k_{\max} = a_1 T^2 + a_2 T + a_3$  | $\begin{align*} a_1 &= 0.0061 \pm 0.0004 \\
a_2 &= -0.108 \pm 0.011 \\
a_3 &= 1.108 \pm 0.065 \end{align*}$ | 0.048 | 0.998 |
|               | $t_s$     | $t_s = a_1 T + a_2$                   | $\begin{align*} a_1 &= -0.135 \pm 0.019 \\
a_2 &= 5.47 \pm 0.26 \end{align*}$ | 0.020 | 0.959 |
| mWeibull$^c$  | $\delta$  | $\delta = a_1 T + a_2$                | $\begin{align*} a_1 &= -0.182 \pm 0.030 \\
a_2 &= 6.56 \pm 0.41 \end{align*}$ | 0.026 | 0.947 |
| Geeraerd$^d$  | $k_{\max}$ | $\ln(k_{\max}) = a_1 T + a_2$       | $\begin{align*} a_1 &= 0.074 \pm 0.007 \\
a_2 &= -0.639 \pm 0.096 \end{align*}$ | 0.008 | 0.982 |

$^a$ Data are mean ± standard error.

$^b$ Not significant ($P < 0.05$).

$^c$ The shape factor ($\rho$) at 12 and 17°C was estimated by interpolation because it did not have a clear trend with temperature.

$^d$ The parameter $t_s$ of the Geeraerd model at 12 and 17°C also was determined by interpolation because it did not have a clear trend with temperature.

FIGURE 2. Observed values and predicted survival curves of Listeria monocytogenes in Katiki during storage at 12°C as generated by the reparameterized Gompertz model (A), the modified Weibull model (B), the Geeraerd model (C), and the radial basis function neural network (D). Dotted lines are ±0.5 log units from the predicted curve. Data points are mean (±standard error) of two independent experiments with two replications each.
FIGURE 3. Observed values and predicted survival curves of *Listeria monocytogenes* in Katiki during storage at 17°C as generated by the reparameterized Gompertz model (A), the modified Weibull model (B), the Geeraerd model (C), and the radial basis function neural network (D). Dotted lines are ±0.5 log units from the predicted curve. Data points are mean (±standard error) of two independent experiments with two replications each.

The prediction capability of the developed RBF NN was compared against that of the three selected models that also were used to predict the survival curves of *L. monocytogenes* under the same temperature conditions. A two-step standard procedure used in predictive microbiology was followed. Initially, the primary models (reparameterized Gompertz, modified Weibull, and Geeraerd models) were fitted to the survival data and the relative kinetic parameters were estimated (Table 1). Next, the kinetic parameters (\(k_{\text{max}}, t_s, \delta, \text{and } p\)) were related to temperature through a secondary first or second order polynomial model (Table 3), and their new values were estimated at 12 and 17°C. The parameters \(N(0), A, \text{and } N_{\text{res}}\) were similar for all four temperatures examined (Table 1), and their mean values were used for validation purposes. Based on the new values of the kinetic parameters, equations 1, 2, and 3 were recalculated and refitted (Figs. 2 and 3). The results revealed that the RBF NN was able to predict the survival of *L. monocytogenes* at 12 and 17°C equally well as did the other three models, as inferred by the comparison of the relevant statistical indices (Table 4), implying that the network had learned the underlying process with high accuracy. The network predictions was considered satisfactory because the difference between observed and predicted values were in the range of ±0.5 log units at both validation temperatures (Figs. 2 and 3). The values of the bias factors were close to unity, indicating good agreement between observations and predictions. Generally, models with \(B_f\) values of 0.9 to 1.0 or 1.0 to 1.05 are considered adequate, whereas those with \(B_f\) values of 0.7 to 0.9 or 1.06 to 1.15 are acceptable (63). The comparison of the accuracy factors revealed that at 12°C the mean difference between predictions and observations was 7.0 and 8.2% for the NN and the statistical models, respectively, whereas at 17°C the differences were 8.4 and 9.0%, respectively. A graphic comparison between observed and predicted counts of *L. mono-
TABLE 4. Comparison of validation indices ($B_f$, $A_f$, GoF) between the reparameterized Gompertz model, modified Weibull model, Geeraerd model, and radial basis function neural network at 12 and 17°C

<table>
<thead>
<tr>
<th>Model type</th>
<th>$B_f$</th>
<th>$A_f$</th>
<th>GoF</th>
</tr>
</thead>
<tbody>
<tr>
<td>rGompertz</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12°C</td>
<td>1.033</td>
<td>1.077</td>
<td>0.3539</td>
</tr>
<tr>
<td>17°C</td>
<td>1.029</td>
<td>1.068</td>
<td>0.4246</td>
</tr>
<tr>
<td>mWeibull</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12°C</td>
<td>0.991</td>
<td>1.092</td>
<td>0.3401</td>
</tr>
<tr>
<td>17°C</td>
<td>1.010</td>
<td>1.107</td>
<td>0.4216</td>
</tr>
<tr>
<td>Geeraerd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12°C</td>
<td>0.996</td>
<td>1.077</td>
<td>0.3133</td>
</tr>
<tr>
<td>17°C</td>
<td>0.978</td>
<td>1.095</td>
<td>0.3560</td>
</tr>
<tr>
<td>RBF NN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12°C</td>
<td>1.035</td>
<td>1.070</td>
<td>0.3137</td>
</tr>
<tr>
<td>17°C</td>
<td>1.028</td>
<td>1.084</td>
<td>0.3956</td>
</tr>
</tbody>
</table>

**cytogenes** at 12 and 17°C is presented in Figure 4. There is scatter around the line of equity (1:1), with the data points of NN performing equally well as do those of the statistical models. However, the predicted values were not scattered uniformly around the line of equity for the entire range of compared values. At 17°C (Fig. 4B), the predicted values were systematically higher than the experimental values, especially at the higher population densities, regardless of the model employed, indicating overprediction by the model.

NNs have been successfully employed in predictive microbiology as an alternative to regression models. However, most of the developed networks have been based on the multilayer perceptron network (MLP) (11, 16, 19, 21, 28, 32, 42, 43, 69, 75), and fewer approaches have been based on general regression NNs (37), genetic algorithms (20, 26), recurrent NNs (13, 14), and hybrid NNs (70). The MLP structure is probably the most widely used NN paradigm and has long proven nonlinear modeling capabilities and performance. The knowledge of the network is stored in the weights connecting the artificial neurons. The massively interconnected structure of the MLP NN provides a great number of these weights and as such a great capacity for storing complex information. The standard learning algorithms for these networks, such as the well-known backpropagation learning scheme, are iterative learning measures. In their simplest form, they often suffer from slow convergence and problems with local maxima in the error surface. Measures taken to address these issues often lead to an increase in the computational complexity of the training algorithm (31). In contrast to the popular MLP structure, the RBF NN provides a flatter architecture because of the way in which they incorporate the nonlinear information. The use of radial activation functions provides a nonlinear method of interpolating between numbers in different regions of the information space (8). One of the main advantages of the RBF structure is the rapid training procedure, usually orders of magnitude faster than that of MLP models but exhibiting none of the training pathologies such as paralysis or local minima problems. An RBF NN is a function based on a built-in distance criterion with respect to a center. One major difference is the use of a local learning strategy by RBF NNs versus global learning in the MLP NNs, resulting in higher accuracy and faster training times.

The results of the present study indicate that an RBF NN can be used successfully as a one-step modeling procedure to determine the behavior of *L. monocytogenes* in Katiki during storage at different temperatures. Based on both statistical indices and graphic plots, the performance of the network for the training and validation data sets was equal to or slightly better than that of the statistical models. A potential practical advantage of the NN approach is that fitting and validation can be performed at the same time by the development of a single model. Compared with their

**FIGURE 4.** Comparison between the observed and predicted populations of *Listeria monocytogenes* in Katiki during storage at 12°C (A) and 17°C (B) as generated by the reparameterized Gompertz model (◇), the modified Weibull model (△), the Geeraerd model (□), and the radial basis function neural network (○).
use in other areas, NNs in the field of food science are still in the early development stage, and the use of NNs in predictive microbiology remains relatively rare. The results of this work could increase the knowledge base for the effectiveness of NN application in predictive microbiology.

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REFERENCES


