Artificial Neural Network-Based Predictive Model for Bacterial Growth in a Simulated Medium of Modified-Atmosphere-Packed Cooked Meat Products

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The data of Devilieghere et al. (*Int. J. Food Microbiol.* ***1999***, *46*, 57−70) on bacterial growth in a simulated medium of modified-atmosphere-packed cooked meat products was processed for estimating maximum specific growth rate μ_{max} and lag phase λ of *Lactobacillus sake* using artificial neural networks-based model (ANNM) computation. The comparison between ANNM and response surface methodology (RSM) model showed that the accuracy of ANNM prediction was higher than that of RSM. Two-dimensional and three-dimensional plots of the response surfaces revealed that the relationships of water activity a_w, temperature T, and dissolved CO_2 concentration with μ_{max} and λ were complicated, not just linear or second-order relations. Furthermore, it was possible to compute the sensitivity of the model outputs against each input parameter by using ANNM. The results showed that μ_{max} was most sensitive to a_w, T, and dissolved CO_2 in this order; whereas λ was sensitive to T the most, followed by a_w, and dissolved CO_2 concentrations.

Keywords: *Artificial neural networks; response surface model; bacterial growth; Lactobacillus sake; modified-atmosphere-packed meat*

INTRODUCTION

Growth-predictive models are currently accepted as informative tools that assist in rapid and cost-effective assessment of microbial growth for product development, risk assessment, and education purposes (*2*−*5*). More recently, predictive microbiology has been used to forecast the growth of spoilage microorganisms in order to study the shelf life of a food product. Specific spoilage organisms are selected for certain food products and used as test organisms. For example, *Photobacterium phosphorium* in modified-atmosphere-packed fresh fish from tempered seawater (*6*); *Lactobacillus sake* in modified-atmosphere-packed cooked meat products (*1*), and *Pseudomonas putida* for chill-stored food products such as milk and fresh meat (*7*). Therefore, the investigation and understanding of the influence of environmental factors such as temperature (T), water activity (a_w), and dissolved CO_2 concentrations on the growth of *L. sake* are useful.

Predictive models for the growth of microorganisms include temperature, pH, and water activity as main growth-determining factors (*4*, *8*). However, other factors can also significantly influence the growth characteristics of the modeled microorganism, such as organic acid concentration and atmosphere. More recently, the atmosphere is gradually being taken as a fourth important growth-determining factor (*1*, *9*−*13*).

In recent studies on microbial growth, response surface methodology (RSM) provided an alternative, useful approach for quantitative assessment of the effects of environmental factors on microbial growth (*1*, *4*, *8*, *14*, *15*). Generally speaking, RSM usually is determined using multiple linear-regression analysis and may require a large number of cross-section parameters for better representation of experimental data. In practice, the widely used RSM is quadratic RSM. Once the RSM is developed, the growth of microorganisms (response) under various conditions within the range of the variables used to develop the model can be constructed. The drawbacks of the RSM arise from the facts that (1) it is developed from linear and quadratic combinations of model variables where linearity may or may not be justified, (2) the collinearity between terms may exist, and (3) the sensitivity analysis of the model output against each input parameter is inconvenient because of the cross-terms. However, it is well-known that the artificial neural network-based (ANN) model, being a highly nonlinear approximator, frequently outperforms first- and second-order RSM, thereby leading to better mapping of nonlinear data (*16*).

The objectives of this study, therefore, were (1) to develop an artificial neural network-based model (ANNM) for predicting the combined effects of temperature (T), water activity (a_w), and dissolved CO_2 concentrations on the maximum specific growth rate and the lag phase of *L. sake* in gas-packed cooked meat products, (2) to compare the prediction accuracy of ANNM and RSM to assess the capability of ANN as a substitute of RSM, (3) to apply the developed ANN model to evaluate the relative importance of the three environmental factors in controlling the growth of *L. sake*, and (4) to employ the ANN to generate response surface of the bacterial growth.

MATERIALS AND METHODS

Experimental Data. The data reported by Devlieghere et al. (*1*) were used to test the ability of the ANN technique in

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modeling the growth of *L. sake* in relation to temperature (denoted as X_1), dissolved CO_2 concentration (denoted as X_2), and water activity (a_w) (denoted as X_3). A multifactorial design arrangement resulted in the total 119 data set under different possible environmental conditions. The ranges of the parameters were $X_1 = 4-12$ °C, $X_2 = 0-2411$ ppm, and $X_3 = 0.962-0.986$.

Response Surface Methodology. The maximum specific growth rate μ_{max} and the lag phase λ were the two response functions investigated in this study. Each of these two responses was fitted with an empirical equation in order to correlate to the independent variables, i.e., three controlling factors. The RSM for μ_{max} or λ (denoted as Y), with quadratic response surface model including three variables X_1, X_2, and X_3 was expressed by Khuri and Cornell (*17*).

$$Y = a_0 + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3}\sum_{j=1}^{3} a_{ij} X_i X_j \qquad (1)$$

where X_1, X_2, and X_3 are the input or controlling variables, with or without codes, which influence the response variable Y; coefficients a_0, a_i ($i = 1, 2, 3$), and a_{ij} ($i = 1, 2, 3$; $j = i, ..., 3$) are the model coefficient parameters. The values of the coefficients are estimated by multiple linear regression analysis by the least-squares method. The terms whose coefficients were nonsignificant were deleted backward, stepwise, and finally, only the terms that had significantly affected the model remained in the equation.

Artificial Neural Networks (ANN). The structure and operation of ANN have been discussed and some modified algorithms were proposed by a number of authors (*18*, *19*). Thus, the structure and the basic principle are not herein described.

In practice, the most widely used neural networks are back-propagation algorithms. Multilayer neural networks with fully interconnected layers are considered in this paper. Layer l consists of $n^l + 1$ neurons, the last neuron being a bias neuron with a constant output of 1.0. If there are P patterns (samples) for neural network training, the principle of back-propagation algorithm can be briefly written as the total input to neuron j in layer l (noninput layer), I^l_j is determined by

$$I^l_j = \sum_i w_{ij}^{l-1,l}\, x_i^{l-1} \qquad (2)$$

where $w_{ij}^{l-1,l}$ represents the connecting weight between neuron j in layer l and neuron i in the next lower layer. The activity level of neuron j is a linear or nonlinear function of its total input:

$$O^l_j = f(I^l_j) \qquad (3)$$

where $f(\cdot)$ denotes a nondecreasing smooth function (generally called transfer function or activation function). The transfer function for the hidden layer is the conventional sigmoidal function with the range of (0, 1), or hyperbolic function with the range of (−1, 1). The linear function is always used in output layer, which assists in accelerating the converged procedure, thereby avoiding false local minima (*20*).

Learning is carried out by iteratively updating the connecting weights to minimize the sum-of-squares error function E, which is defined as:

$$E = \frac{1}{2}\sum_{p=1}^{P}\sum_{j=1}^{n^o}(O^o_{p,j} - d_{p,j})^2 \qquad (4)$$

where n^o is the number of outputs; P is the input patterns or samples; and $O^o_{p,j}$ and $d_{p,j}$ are scaled network actual and desired output values, respectively.

As most combinations of weights produce a different error, an error surface exists as a function of the connection weights. This error surface generally has a large number of local minima as a result of the large number of permutations of the weights that leave the neural network's input/output function unchanged. The aim of training is to find a set of weights that will minimize the error function.

Initially, the weights are small, arbitrary values. As training (learning) progresses, the weights are updated systematically using a learning rule, usually error back-propagation algorithm. The weight updating equation generally takes the form:

$$w_{ij}(t+1) = w_{ij}(t) + \eta\Delta w_{ij}(t+1) + \alpha\Delta w_{ij}(t) \qquad (5)$$

where α and η are the momentum and learning rate, respectively. In eq 5, the size of the steps decreasing the error surface is thus determined using the learning rate η and the momentum α. There are several methods for finding the weight increment, Δw_{ij}, of which the gradient descent method is frequently used. The gradient descent method results in weights being changed in the direction of steepest descent down on the error surface:

$$\Delta w_{ij} = \frac{\partial E}{\partial w} \qquad (6)$$

ANN generally learns an approximation to the underlying rules governing a relationship. Consequently, it is not well suited to learning deterministic input-output relationships, as it is usually not able to perform exact mappings. However, because ANN has the ability to learn the underlying relationships between inputs and outputs, it is well suited to modeling natural systems, where complex relationships exist between the inputs-outputs, especially when data are incomplete or noisy (*21*).

Data Preprocessing. The RSM belongs to the class of model-driven method, implicating that the parameters are estimated according to the predetermined equation. The modeled results are usually not influenced, or only slightly influenced, by the data preprocessing. However, ANN belongs to the class of data-driven approaches, so that the underlying relationship between the inputs and outputs is mapped by iterative learning of the training data sets. In ANN modeling, all the input/output variable data should be normalized into the range of (0, 1) or (−1, 1) for sigmoidal or hyperbolic transfer function, respectively (*22*). One way in scaling input/output variable in interval $[\xi_1, \xi_2]$ corresponding to the range of the transfer function is

$$x_{norm} = \xi_1 + (\xi_2 - \xi_1)\frac{(x - x_{min})}{(x_{max} - x_{min})} \qquad (7)$$

where the x and x_{norm} are the original and its corresponding normalized value, and x_{max}, x_{min} are the maximal and minimal values of each variable over the whole data set. Furthermore, it is more preferable to normalize the data between slightly offset above-mentioned values such as 0.1 and 0.9 rather than 0 and 1, thereby letting $\xi_1 = 0.1$ and $\xi_2 = 0.9$ for sigmoidal transfer function.

Evaluation Criteria. To compare the fitting/training and prediction accuracy of ANN with RSM, the following evaluation criteria were employed (*23*, *24*).

Root-Mean-Squares Error (RMSE). The *RMSE* is calculated between the desired and actual network output and then averaged across all data. It can provide us information about how consistent the model would be in the long run. The formula of *RMSE* is

$$RMSE = \sqrt{\sum_{p=1}^{P}\sum_{j=1}^{n^o}(O^o_{p,j} - d_{p,j})^2/Pn^o} \qquad (8)$$

Average Absolute Percentage Error (AAPE). It is a nondimensional quantity that permits an accurate quantitative comparison among several attempted models. The *AAPE* is

$$AAPE = \frac{\sum_{p=1}^{P}\sum_{j=1}^{n^o}|(O_{p,j}^o - d_{p,j})/d_{p,j}|}{Pn^o} \quad (9)$$

Average Absolute Error (AAE). It is another index, replacing *AAPE* in situations when the absolute of target value is small, that permits an accurate quantitative comparison among several attempted models. The *AAE* is

$$AAE = \frac{\sum_{p=1}^{P}\sum_{j=1}^{n^o}|O_{p,j}^o - d_{p,j}|}{Pn^o} \quad (10)$$

AAE and *RMSE* rely on the average actual error value indicating the overall error, which exists between the observed and predicted values. These indices directly indicate the predicted absolute error. In general, *RMSE* is more suitable than *AAE* for errors, which are all greater than 1 or less than 1, whereas *AAE* can be used in either case. In contrast, the absolute errors are not immediately interpretable, thus, the absolute errors may not be of as much interest as the relative errors in prediction. *AAPE* is a relative deviation, which is a rather reasonable expression to indicate the predictive accuracy. However, when the observed values are small, it is improper to use *AAPE*.

Determination Coefficient (R^2). The determination coefficient represents the relation between desired and model actual outputs. A high R^2 alone cannot indicate a good model unless the intercept and slope of the best-fit model approach 0 and 1, respectively. The R^2 is calculated as

$$R^2 = 1 - \sum_{p=1}^{P}\sum_{j=1}^{n^o}(O_{p,j}^o - d_{p,j})^2 / \sum_{j=1}^{n^o}\sum_{p=1}^{P}(O_{p,j}^o - \overline{d}_j)^2 \quad (11)$$

where $\overline{d}_j = (1/P)\sum_{p=1}^{P} d_{p,j}$.

Sensitivity Analysis Criteria. Neural network can conduct a sensitivity analysis of the inputs to a neural network (*25*). The sensitivity is defined as the ratio between the error with omission and the baseline error, and ranks the variables in the order of importance. It often identifies variables that can be safely ignored in subsequent analysis, as well as key variables that must always be retained. The sensitivity of an input variable against the output variable can be assessed through the following criteria.

Variable Sensitivity Error (VSE). The *VSE* indicates the performance of the developed network if that variable is unavailable. An important variable has a high *VSE*, thus indicating that the network performance badly deteriorates if it is not present.

Variable Sensitivity Ratio (VSR). The *VSR* is a relative indication reporting the ratio between the *VSE* and the error of the network when all variables are available. If the *VSE* is one or less, the network actually performs better if a variable is omitted entirely. We can rank the variables in order of importance according to their *VSR*.

ANN Geometry. When building a neural network, a key design decision is to determine the number of layers and then the responsible neurons in each layer. It has been shown that ANN with one hidden layer can approximate any continuous function (*26*), and one hidden layer is thus used in this study. Furthermore, Najjar et al. (*27*) and Statsoft (*25*) suggested two iterative methods, the so-called construction method and destruction method, respectively, for determining the appropriate number of neurons in the hidden layer. In general, a smaller network executes faster, trains faster, and generalizes more accurately to untrained data. Increasing the number of hidden neurons can enhance the modeling power of the network, in that it can model a more convoluted, complex underlying function. However, this also makes it large, thus being more difficult to train, rendering it slower to operate, and thereby resulting in over-fitting.

In contrast, decreasing the number of hidden neurons has an opposite effect. In fact, the prediction accuracy using a sum-of-squares error function of the ANN with the optimum number of hidden neurons reaches its minimum, at which point the overall error will decrease much more slowly, thus, the network achieves an optimal balance between over-training and under-training. In other words, the number of the hidden layer neurons should be chosen as small as possible if only the ANN satisfies the predetermined accuracy, so that over-training is unlikely to occur.

ANN Training, Verifying, and Testing. The 119 data were split into three sets: 99 data training set, 10 data verification set, and 10 data test set. To avoid any bias, verification set and testing set were all selected randomly from the entire database. The STATISTICA Networks (*25*) was used in this study. A constant momentum of α=0.3 and the learning rate of μ=0.1 (default values) were used to train the network. The condition used to determine whether the learning process stopped or the training error was less than the predetermined value in this study was 0.001 h^{-1} for variable μ_{max} and 22 h for λ.

Table 1. Evaluation Criteria of Predictive Models for Maximum Specific Growth Rate μ_{max}

	RMSE	AAE	adjusted R^2
RSM model	0.013	0.0098	0.926
ANN model	0.011	0.0090	0.943

Table 2. Evaluation Criteria of Predictive Models for Lag Phase λ

	RMSE	AAPE	adjusted R^2
RSM model	6.88	5.39	0.966
ANN model	6.70	5.13	0.972

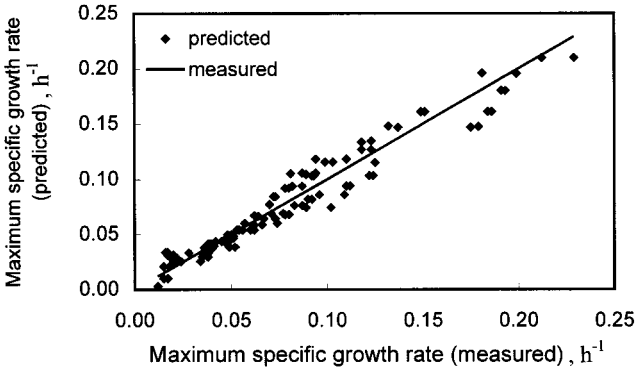

Figure 1. Comparison between the predicted and measured maximum specific growth rate μ_{max} (h^{-1}) for *L. sake.*

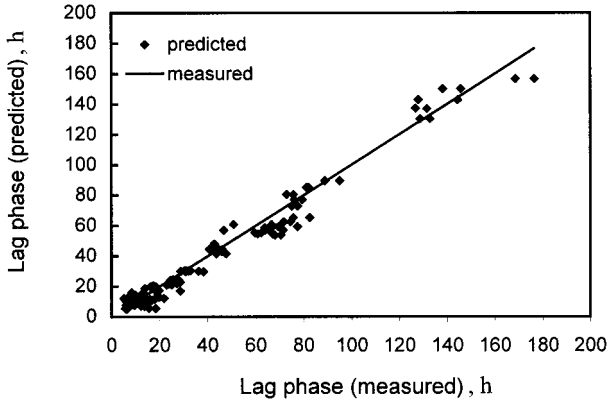

Figure 2. Comparison between the predicted and measured lag phase λ (h) for *L. sake.*

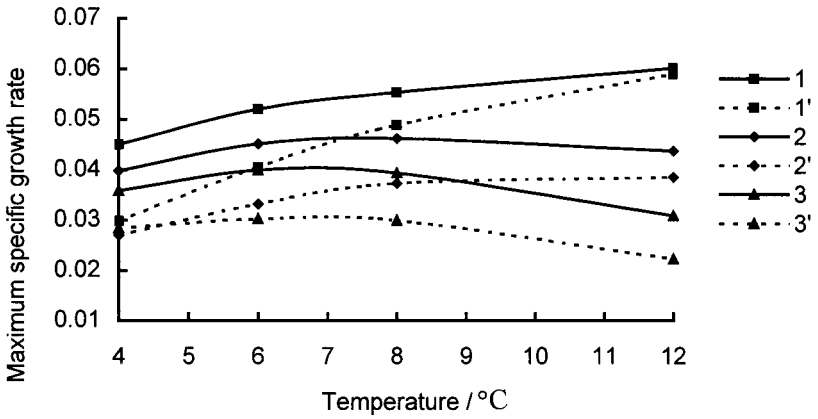

Figure 3. Influence of temperature on the prediction of μ_{max} (h^{-1}) at low water activities (here $a_w = 0.965$) and various dissolved CO_2 levels (1, 0 ppm; 2, 1000 ppm; and 3, 2000 ppm) using ANN predictive model (solid line) and RSM (dashed line), respectively.

Table 3. Sensitivity Analysis of Variables for Maximum Specific Growth Rate μ_{max}

		variables		
		temperature (°C)	dissolved CO_2 (ppm)	water activity a_w
training	rank	2	3	1
	VSE	0.0339	0.0189	0.0390
	VSR	2.365	1.322	2.722
verification	rank	2	3	1
	VSE	0.0432	0.0213	0.0447
	VSR	4.210	2.079	4.353

Table 4. Sensitivity Analysis of Variables for Lag Phase λ

		variables		
		temperature (°C)	dissolved CO_2 (ppm)	water activity a_w
training	rank	1	3	2
	VSE	36.2390	7.9084	25.3079
	VSR	5.506	1.202	3.8450
verification	rank	1	3	2
	VSE	25.2306	6.7885	9.4778
	VSR	3.7933	1.021	1.425

RESULTS AND DISCUSSION

ANN-based models were compared with response surface models in terms of predictive accuracy, relative importance, and effect of input variables on maximum growth rate.

Predictive Accuracy. Using the sigmoid transfer function throughout experiments, it was found that the network structure 3−2−1 was reasonable in ANN-based models as far as the training and verification accuracies and structure compaction were concerned. With this small number of hidden layers as well as close errors of training, verification and test subsets warrant that over-learning has not occurred (*25*).

Tables 1 and 2 show the evaluation criteria of RMSE derived from regression analysis and ANNM for the maximum specific growth rate μ_{max} and lag phase λ, respectively. The ANN-based predictive models are in good agreement with the experimentally measured responses to a greater extent than those of RSM models. Figure 1 shows the comparison between the experimental μ_{max} and its predicted value by ANN-based model. Figure 2 shows the comparison between the experimental λ and the corresponding values predicted by ANN-based model for the entire data sets. These results are similar to those using the extended Ratkowsky models reported by Devlieghere et al. (*1*), except for smaller deviations at $\mu_{max} < 0.06$ h^{-1} and $\lambda < 50$ h.

Relative Importance of Input Variables. The training set and verification set of the entire 109 data set (another 10 data are used for testing) utilized to develop the network were used to calculate the variable sensitivity error (VSE) and variable sensitivity ratio (VSR). The *VSE* and *VSR* for the output variable μ_{max} and λ with respect to temperature (T), water activity (a_w), and dissolved CO_2 concentrations are shown in Tables 3 and 4, respectively. For the maximum growth rate μ_{max}, the water activity a_w is the most sensitive parameter in the model, followed by T and dissolved CO_2 concentrations (Table 3); whereas the lag phase λ is most sensitive to temperature T, followed by a_w, and then dissolved CO_2 concentrations (Table 4).

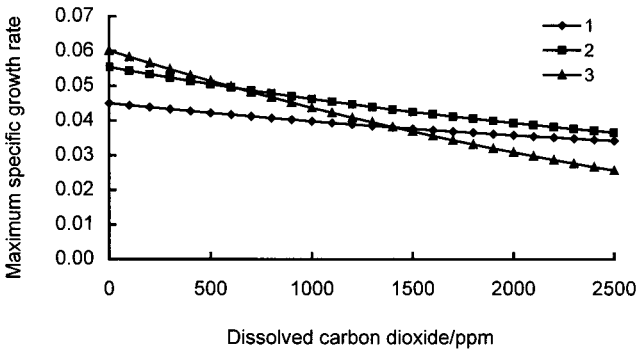

Figure 4. Influence of dissolved CO_2 on the prediction of μ_{max}(h^{-1}) at median temperature (here $T = 8°$ C) and various water activities (1, $a_w = 0.965$; 2, $a_w = 0.975$; 3, $a_w = 0.985$) using the ANN predictive model.

Effect of Input Variables on Maximum Growth Rate (μ_{max}). Figures 3, 4, and 5 show the effect of each input variable on the maximum growth rate μ_{max}. On the basis of the data shown in Figure 3, it is concluded that at a dissolved CO_2 concentration lower than1000 ppm, μ_{max} increases with an increase in temperature. However, at higher dissolved CO_2 of 2000 ppm, when the temperature rises, the maximum growth rate μ_{max} increases first, and then decreases, with the turning-point at about 7 °C. This decrease was regarded as "illogical" by Devlieghere et al. (*1*). Because the RSM lines in Figure 3 are in good agreement with their counterpart in Figure 1 of Devlieghere et al. (*1*), the illogical prediction by RSM at low water activity is likely to be the fact and not necessarily illogical.

Figure 4 shows that when water activity is high, the μ_{max} decreases quicker than at lower water activities

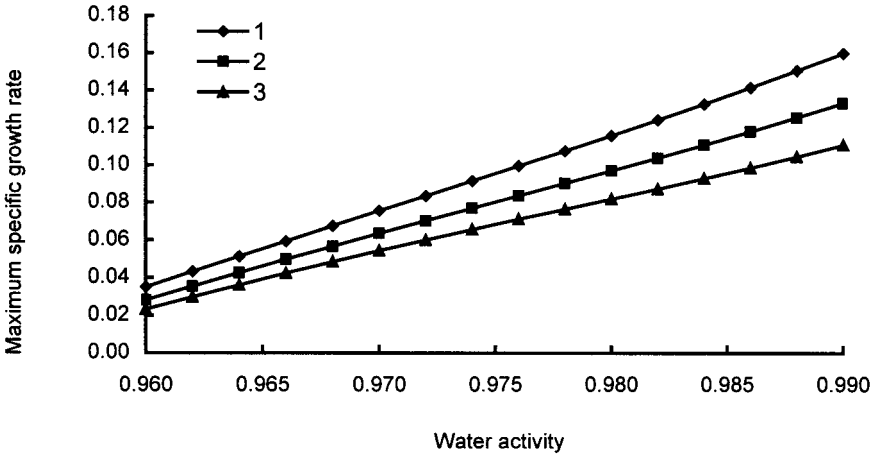

Figure 5. Influence of water activity on the prediction of μ_{max} (h^{-1}) at medium dissolved CO_2 (here 1000 ppm dissolved CO_2) and various temperatures (1, $T = 4$ °C; 2, $T = 8$ °C; 3, $T = 12$ °C) using the ANN predictive model.

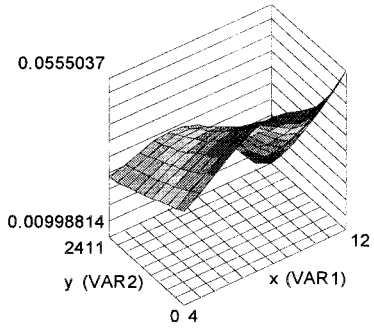

Figure 6. Variation of the maximum specific growth rate μ_{max} (h^{-1}) with dissolved CO_2 (VAR2) and temperature (VAR1) at $a_w = 0.965$.

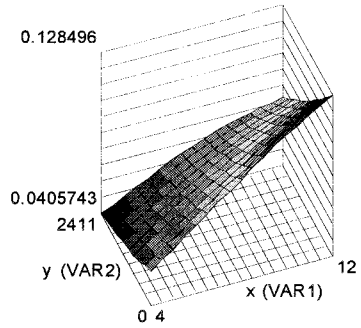

Figure 7. Variation of the maximum specific growth rate μ_{max} (h^{-1}) with dissolved CO_2 (VAR2) and temperature (VAR1) at $a_w = 0.975$.

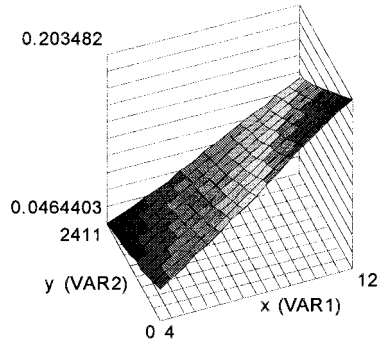

Figure 8. Variation of the maximum specific growth rate μ_{max} (h^{-1}) with dissolved CO_2 (VAR2) and temperature (VAR1) at $a_w = 0.985$.

as the dissolved CO_2 increases. It is interesting to note that at $a_w = 0.985$ and 12 °C, μ_{max} with dissolved CO_2 of 2000 ppm has reduced to about the same level as that at $a_w = 0.965$ (Figure 3). Within the temperature range investigated (4∼12 °C) and at dissolved CO_2 of 1000 ppm, μ_{max} increases with increase in water activity (Figure 5).

Nine 3-D plots were generated to represent variation of μ_{max} at various T, dissolved CO_2, and a_w values. Among them, three plots for low, medium, and high a_w (0.965, 0.975, 0.985) are illustrated in Figures 6, 7 and 8, respectively.

Following are the results of these 3-D plots made by ANN (*25*): (1) At $a_w = 0.965$ (Figure 6), the response surface is more complicated than those at other a_w values, which Devlieghere et al. (*1*) regarded illogical; and at low temperatures, the response slightly increases as the dissolved CO_2 decreases; in contrast, at high temperatures, μ_{max} more rapidly increases with decrease in dissolved CO_2. (2) At medium or high a_w(Figures 7 and 8), the response surfaces are simply quadratic, showing that the higher the temperature and the lower the dissolved CO_2, the greater the μ_{max}. (3) Regardless of the temperature, the higher the a_w, the greater the μ_{max}. At $T = 12$ °C and dissolved $CO_2 = 0$ ppm, the μ_{max} was 0.060, 0.128, and 0.204 at a_w=0.965, 0.975, and 0.985, respectively. (4) In general, as temperature became low, the effects of dissolved CO_2 at medium or high a_w on the growth rate were inclined to reduce.

The nonlinear complexity in the model at low water activity is clearly shown in Figure 6 in comparison with Figures 7 and 8 at higher water activity. For nonlinear cases such as this, ANN is obviously more appropriate to apply than linear RSM. Accordingly, the interpretation of models should be different. An advantage of the ANN-based predictive model over an RSM model is that ANN is a powerful tool for constructing microbial prediction models. When information on additional environmental factors becomes available, the ANN model is recommended to extend by adding neurons and/or layers for predicting the shelf life.

CONCLUSIONS

This paper demonstrated that models could be constructed to predict bacterial growth rate and lag phase of modified-atmosphere-packed meat products more accurately by using ANN than RSM. This can be attributed to the ANN's ability to develop nonlinear mapping of data. Sensitivity of the ANN model revealed

that the water activity was the most important controlling factor affecting the maximum specific growth rate, μ_{max}, of the *L. sake*, followed by temperature, and then dissolved CO; however, the lag phase λ was most affected by T, followed by a_w, and then dissolved CO_2 concentration. These factors are also synergistic in the effect on the shelf life of cooked meat products. Outstanding antimicrobial activity of CO_2 was observed at low water activity and temperatures above 7 °C, which is about equal in the level at higher water activity.

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