J. Dairy Sci. 90:3118–3125
doi:10.3168/jds.2006-591

Estimation of Storage Time of Yogurt with Artificial Neural Network Modeling

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ABSTRACT

Changes in the physical, chemical, and microbiological structure of yogurt determine the storage and shelf life of the product. In this study, microbial counts and pH values of yogurt during storage were determined at d 1, 7, and 14. Simultaneously, image processing of yogurt was digitized by using a machine vision system (MVS) to determine color changes during storage, and the obtained data were modeled with an artificial neural network (ANN) for prediction of shelf life of set-type whole-fat and low-fat yogurts. The ANN models were developed using back-propagation networks with a single hidden layer and sigmoid activation functions. The input variables of the network were pH; total aerobic, yeast, mold, and coliform counts; and color analysis values measured by the machine vision system. The output variable was the storage time of the yogurt. The modeling results showed that there was excellent agreement between the experimental data and predicted values, with a high determination coefficient (R^2 = 0.9996) showing that the developed model was able to analyze nonlinear multivariant data with very good performance, fewer parameters, and shorter calculation time. The model might be an alternative method to control the expiration date of yogurt shown in labeling and provide consumers with a safer food supply.
Key words: artificial neural network, yogurt, machine vision, prediction of shelf life

INTRODUCTION

Yogurt is a semisolid fermented milk product consumed in most parts of the world. Changes in the physical, chemical, and microbiological structure of yogurt determine the storage and shelf life of the product. Alteration of these properties causes color, aroma, and texture deterioration of yogurt, which are considered important quality criteria by consumers.

Artificial neural networks (**ANN**) are new information processing techniques offering solutions to problems that have not been clearly formulated. Much of the excitement surrounding neural networks is their unique ability to learn by experience. In the past few years, neural networks have shown increased power over many other statistical methods when solving nonlinear prediction problems (Bochereau et al., 1992). Artificial neural networks are general nonlinear models based on a simplification of human brain function; more than other modeling strategies, they have the capability to internally self-adapt and encapsulate complex nonlinear relationships between input and output variables without the need for an a priori rigid model structure (Hornik et al., 1989; Thibault and Grandjean, 1992). As in nature, the network function is determined largely by the connections between elements. A neural network can be trained to perform a particular function by adjusting the values of the connections (weights) between the elements. Commonly, neural networks are adjusted or trained so that a particular input leads to a specific target output.

There have been many applications of ANN reported for the interpretation of images in the agri-food industry (Bishop, 1994). Fuzzy logic, genetic algorithms, and similar regression analyses have been used for prediction of shelf life of food, but generally the best results have been obtained by using ANN (Doganis et al., 2006). Artificial neural networks have been used, successfully, as a modeling tool in several food processing applications such as sensory analysis and quality control (color analysis, textural evaluation, human preferences, and so on), classifications, microbiology, and drying applications (Ni and Gunasekaran, 1998; Edwards and Cobb, 1999; Farkas et al., 2000; Hussian et al., 2002).

Increasing demand for fast, reliable, and objective techniques to determine shelf life of food products with high quality and safety standards encourages scientists to use image analysis techniques for food quality evaluation (Park and Chen, 2000; Kavdir and Guyer, 2002; Brosnan and Sun, 2004; Sun and Du, 2004). Ni and Gunasekaran (1998) and Brosnan and Sun (2004) reviewed the use of computer vision technology for food

Received September 13, 2006.
Accepted February 23, 2007.
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analysis. In image analysis for food products, color is an influential attribute of visual information and a powerful descriptor of measurement. Research shows that color has been successful in classifying a variety of food products (Brosnan and Sun, 2004; Sun, 2004). Besides the use of machine vision systems for inspection and grading of fruits and vegetables (Brosnan and Sun, 2004), it has been used routinely in the quality assessment of meat, cheese, and pizza (McDonald and Chen, 1990; Gerrard et al., 1996; Jamieson, 2002). Analysis of the characteristics of Cheddar and Mozzarella cheeses with machine vision during cooking gave promising results, suggesting that the method provided an objective and easy approach for analyzing the functional properties of cheese (Wang and Sun, 2002). Ni and Gunasekaran (1998) developed an image-processing algorithm to recognize individual cheese shreds and automatically measure the shred length.

The objective of this study was to determine color changes during storage of set-type whole-fat and low-fat yogurts using a machine vision system and to develop a prediction model using ANN for prediction of shelf life of yogurt.

MATERIALS AND METHODS

Raw Material

Commercial yogurt samples were obtained from a local market in Isparta (southwestern Turkey). The samples included plain, set-type whole-fat (3% fat) and low-fat (1.5% fat) yogurt and were stored in the refrigerator (4 to 7°C) for 14 d. Analyses were performed during storage on d 1, 7, and 14.

Chemical Analyses

Measurements of pH values of the yogurt samples were performed at 17 to 20°C using a pH meter (Inolab WTW, Measurement System, Fort Myers, FL). Fat content was measured by the Gerber method (British Standards Institution, 1955).

Microbiological Analyses

Total aerobic counts (**TAC**) were determined on plate count agar (Merck, Darmstadt, Germany) incubated at 32°C for 3 d. Coliform, yeast, and mold counts of yogurts were determined using the pour-plate method. Coliform counts (**CC**) were estimated using violet red bile agar (Merck) incubated at 37°C for 48 h. Yeast and mold counts (**YMC**) were carried out on potato dextrose agar (Merck) incubated at 25°C for 5 d.

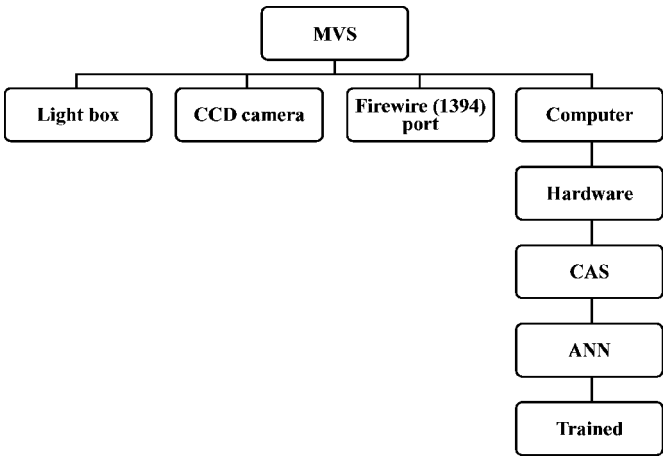

Figure 1. Machine vision system used for color analysis of yogurt. MVS = machine vision system; CAS = color analysis software; CCD charge-coupled device camera; ANN = artificial neural network.

Machine Vision System

Hardware. The machine vision system described by Demir et al. (2002) and Balaban et al. (1997) was used as an alternative for color measurements of yogurt samples. Images were digitized using pixels with information on the levels of 3 primary colors (RGB: red, green, and blue) for an image. This system can give a complete description and amount of all the colors present in the samples with different sizes, shapes, surface textures, and colors. The hardware consisted of a light box, digital color video camera, and computer. The light box was used to standardize illumination and comprised an aluminum framework covered by 100% white safety glazing fabrics. The light box had top and bottom lighting with 2 fluorescent lights (illuminant D50, simulating noon summer sun; General Electric, Cleveland, OH) inside the box. Three yogurt samples were placed in the light box with the top lighting, and the door of the light box was closed. Yogurt samples were observed on d 1, 7, and 14 with a charge-coupled device (**CCD**) color video camera (Sony DFW-V500, Sony Corp., Tokyo, Japan) with 64-bit color, 525 lines, and a horizontal resolution of >460 lines. During image acquisition a standardized color tile was placed next to the yogurt samples in the light box. Images of samples were captured and saved for further color analysis (Luzuriaga et al., 1997). A schematic presentation of the machine vision system is shown in Figure 1.

Software. A color analysis program called Color Expert, running under the Microsoft Windows XP environment, was used to extract color information from a color image. The user interface program was written in Microsoft Visual Basic professional (version 6.0, Microsoft Inc., Redmond, WA), and also uses the Pinnacle Graph-

ics Server (version 5.0, Bits Per Second Ltd., Brighton, UK), and the Formula One spreadsheet from Visual Tools (version 4.1, Visual Components, Lenexa, KS; Luzuriaga et al., 1997). Color data from images of yogurt samples were analyzed by looking at every pixel. The system included a database that stored reference values to correctly evaluate yogurt quality, and saved all results from the analysis in one spreadsheet.

ANN Modeling

The network was adjusted based on a comparison of the output and the target, until the network output matched the target. Typically many such input/target output pairs are used to train a network (Demuth and Beale, 2001). Batch training of a network proceeds by making weight and bias changes based on an entire set (batch) of input vectors. Incremental training changes the weight and biases of a network as needed after presentation of each individual input vector. Incremental training is sometimes referred to as online or adaptive training.

In this study, Levenberg-Marquardt back-propagation (**BP**) network training was applied until the evaluation standard was reached (Demuth and Beale, 2001). These networks are most widely used and are considered the workhorses of ANN (Rumelhart et al., 1986). A BP network is a multilayer perceptron consisting of 1) an input layer with nodes representing input variables, 2) an output layer with nodes representing the dependent variables (i.e., what is being modeled), and 3) one or more hidden layers containing nodes to help capture the nonlinearity in the data. Using supervised learning (with the error correction learning rule), these networks can learn the mapping from one data space to another using examples. The term "back-propagation" refers to the way the error computed at the output side is propagated backward from the output layer, to the hidden layer, and finally to the input layer (Hassoun, 1995).

A neural network model consists of an input layer, an output layer, and one or more hidden layers. In this study, the ANN was designed with only one hidden layer (Figure 2). One hidden layer is usually sufficient to approximate any continuous nonlinear function, although more complex networks must be used in special applications (Bucinski et al., 2004).

The function of the neuron is schematically shown in Figure 2. The inputs (y_i) into a neuron are multiplied by their corresponding connection weights (w_{ij}) and summed:

$$x_j = \sum_{i=1} w_{ij} \cdot y_i \qquad [1]$$

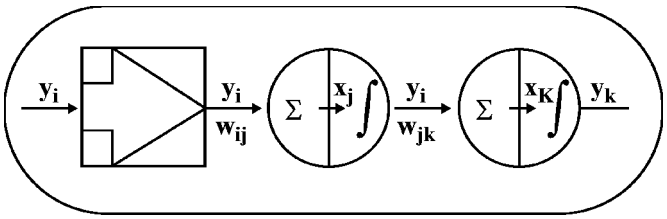

Figure 2. Detail of the function of a neuron. The inputs (y) into a neuron are multiplied by their corresponding connection weights (w) and summed. This sum is then transformed through the sigmoid function to produce a single output that may be passed on to other neurons.

This sum is then transformed using the sigmoid function to produce a single output, y_j, which may be passed on to other neurons:

$$y_j = f(x_j) = \frac{1}{1 + e^{-x_j}} \qquad [2]$$

In the same way, the y_k (prediction values) were calculated taking the corresponding output values of the previous hidden layer neurons (y_j) as inputs and multiplying them by the connection weights w_{jk}.

In the training step, the connection parameters, w, were adjusted such that, for the given input data set, the ANN-predicted output data set matched with the real output data set. At the beginning of the training phase, the network connection weights were random values between 0 and 1. For the given set of inputs to the network, the response of each neuron in the output layer (y_k) was then calculated and compared with the corresponding real output response (r_k). Then, the prediction error associated with the output response (E_k) was computed according to

$$E_k = \frac{1}{2} \sum_k (r_k - y_k)^2 \qquad [3]$$

The weights were adjusted to reduce prediction errors through a BP algorithm where E_k was back-distributed to the previous layers across the network. The optimization of the connection weights was performed by minimizing the error according to

$$w_{jk} = w_{jk}^0 - \mu \cdot \frac{\partial E_k}{\partial w_{jk}} \qquad [4]$$

where w_{jk}^0 is the initial connection weight and μ is the learning coefficient. This coefficient controls the degree by which the connection weights were modified during the training phase. In this study, model selection was

completed for the training set by fixing the learning coefficient and momentum to 0.001 and 0.1, respectively. Then, the trained networks were used to run a set of test data.

Solving Eq. [4] leads to

$$w_{jk} = w_{jk}^2 - \mu \cdot (r_k - y_k) \cdot y_k \cdot (1 - y_k) \cdot y_j \qquad [5]$$

for the connection weights corresponding to the output layer or for the connection weights corresponding to the hidden layer:

$$w_{ij} = w_{ij}^0 - \mu \cdot \sum_k (r_k - y_k) \cdot y_k \cdot (1 - y_k) \cdot w_{jk} \cdot y_j \cdot (1 - y_j) \cdot y_i \qquad [6]$$

Logarithmic sigmoid transfer function was used as the activation function for hidden and output layers. The development of the ANN model involved 2 basic steps, training/learning and testing/validation. During the training stage, the network used the inductive-learning principle to learn from a set of examples called the training set. Input variables of the ANN model were included microbial counts (TAC, YMC, CC), pH values, and obtained color data of yogurt during storage from the machine vision system, which was the color index value performed by the color analysis program looking at each pixel of the input yogurt images.

Out of 30 cases in the prediction time data set, 22 cases (~73%) were used for training/testing and 8 cases (~27%) were used for validation, randomly (Figure 3).

Normalized values in the range of 0 to 1 were used as input and output values. Normalization is a transformation performed on a single data input to distribute the data evenly and scale it into an acceptable range for the network. In normalizing data, the goal is to ensure that the statistical distribution of values for each net input and output is roughly uniform. In addition, the values should be scaled to match the range of the input neurons. Therefore, each input should be normalized in addition to the transformations performed on network inputs.

Proximity of the correlation coefficient to 1 and closeness of the mean square error to 0 shows the efficiency of the shelf life-prediction ANN models. The input layer had 16 neurons. The hidden layer nodes were chosen according to ANN performance. Training was continued until 10,000 epochs had been executed. For a given set of inputs to the network, the response to each neuron in the output layer was calculated and compared with the corresponding desired output response. Different ANN models were developed by using 20 different neurons in 1 hidden layer. The optimum model result was obtained in a hidden layer with 5 neurons; this model is shown in Figure 4.

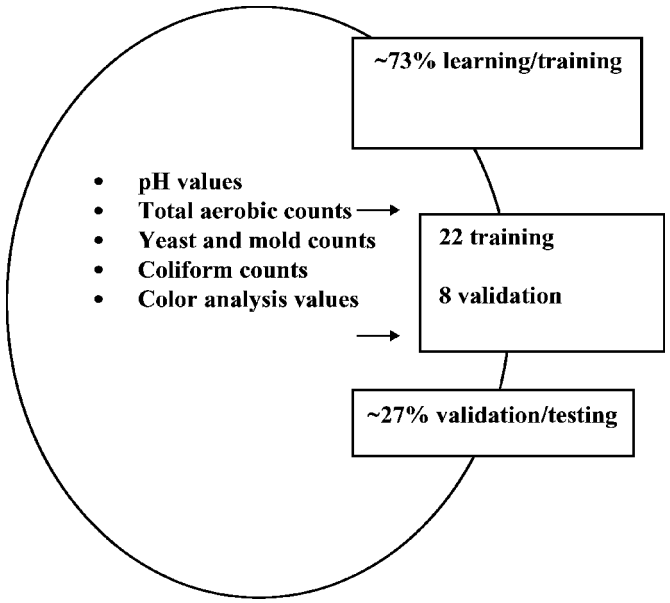

Figure 3. In the developed model, ~73% of the parameters were used for training and ~27% for validation.

Statistical Analysis

The degree of association between pH and microbial counts upon storage was determined by using PROC GLM of SAS (SAS Institute Inc., Cary, NC). Separation of the means ($P < 0.05$) was accomplished by Duncan's multiple range test. All experiments and analyses were replicated 3 times. All analyses within experimental replications were performed in duplicate.

RESULTS AND DISCUSSION

The extent of growth of certain classes of microorganisms and changes in sensory parameters are widely used to mark end of shelf life of foods (Muir, 1996; Lewis and Dale, 2000). In this study, pH, microbial counts (TAC, YMC, CC), and color data were used to mark shelf life (d 1 through 14) of set-type whole- and low-fat yogurts. Then, the ANN model was developed with the obtained data to predict the shelf life of yogurt.

Changes in pH and microbial counts during storage are shown in Table 1. Although the pH of low-fat yogurt did not change significantly ($P > 0.05$), the pH of whole-fat yogurt decreased from 4.20 to 3.76 during storage ($P < 0.05$). Total aerobic plate counts in low-fat yogurt samples increased by 2 log cycles from 3.43 to 5.12 log cfu/g after 14 d of storage. Total plate counts were lower

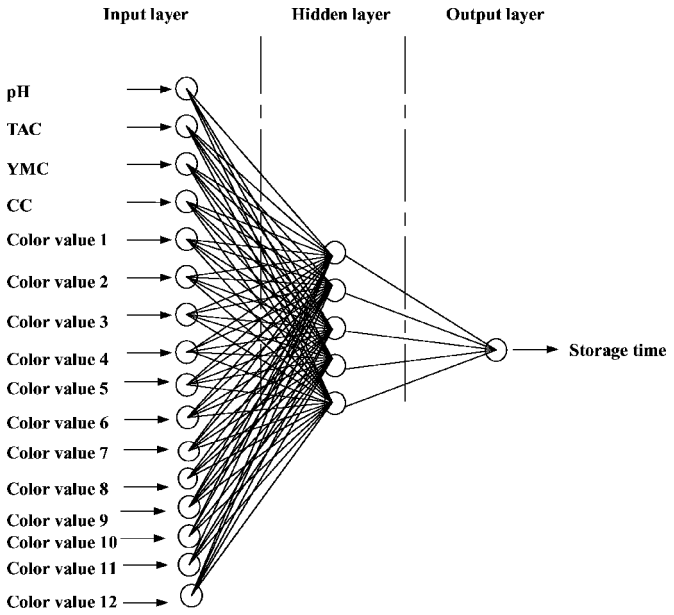

Figure 4. Selected neural network structure: 16 inputs were used for the artificial neural network (ANN): 12 inputs corresponding to color blocks resulting from machine vision system analysis; 1 input corresponding to pH values; 3 inputs corresponding to total aerobic count (TAC), yeast and mold counts (YMC), and coliform counts (CC) resulting from microbial analysis. For the ANN used to predict the shelf life, 1 output was used and the output value was the "shelf-life".

for whole-fat yogurt samples, possibly due to the decrease in pH during storage. Yeasts and molds counts of low-fat yogurt increased during storage with counts ranging within 2 log cycles (from 3.45 to 5.63 log cfu/g; $P < 0.05$), whereas YMC became undetectable for whole-fat yogurts after d 7. Flavor defects in yogurt, quarg, and (initially) yeast-free labneh have been reported to develop when counts of yeasts and molds reach levels of 10^5 cfu/g (Tamime and Robinson, 1999; Kadamany et al., 2003). The coliform counts of low-fat yogurt were high on d 1 (2.58 log cfu/g). Coliforms were present at a level of 1.26 log cfu/g in whole-fat yogurt and decreased progressively during storage, with growth becoming undetectable on the d 7 of storage, presumably due to the inhibitory effect of increased acid production (Tamime and Robinson, 1999). The presence of coliforms in the samples is indicative of postpasteurization contamination at one or more stages during processing.

The color data measured by the machine vision system were reported as a percentage of the total area of the surface by a given color block (data not shown) as described in Luzuriaga et al. (1997). There was an increase in the percentage of the total area of color blocks having color of pale-greenish-yellow, grayish-yellow, light grayish-green, and yellowish-green, out of the 4,096 color blocks from d 1 to 14. The color block with grayish-greenish yellow color was dominant in both whole- and low-fat yogurts at the end of storage. The presence of these colors is associated with microbial spoilage of the food product. The color analysis data were parallel to pH and microbial count data.

The development of the ANN model involved 2 basic steps, training/learning and testing/validation. During the training stage the network used the inductive-learning principle to learn from a set of data called the training set. Out of 30 cases in the prediction time data set, 22 cases (~73%) were used for training/testing and 8 cases (~27%) were used for validation, randomly (Figure 3). Sixteen inputs were used for ANN: 12 inputs corresponding to color blocks resulting from machine vision analysis; 1 input corresponding to pH values; and 3 inputs corresponding to TAC, YMC, and CC resulting from microbial analysis. For the ANN used to predict the shelf life, 1 output was used and the output value was the "shelf-life" (Figure 4).

Determining the optimal ANN topology consisted of selecting the number of neurons that gave a minimum final error in a minimal number of iterations during

Table 1. The pH measurements and microbial counts of low-fat and whole-fat yogurt cultures during storage for 1, 7, and 14 d[1]

Storage day	Yogurt samples	pH	Total aerobic counts, log (cfu/g)	Yeast and mold counts, log (cfu/g)	Coliform count, log (cfu/g)
1	Low fat	4.41 ± 0.01[a]	3.43 ± 0.07[a]	3.45 ± 0.45[a]	2.58 ± 2.11[a]
	Whole fat	4.20 ± 0.16[b]	1.72 ± 0.16[b]	1.07 ± 1.19[b]	1.26 ± 1.01[b]
7	Low fat	4.16 ± 0.01[c]	5.04 ± 0.19[c]	2.96 ± 1.47[c]	1.06 ± 0.24[c]
	Whole fat	3.86 ± 0.03[b]	1.95 ± 0.99[d]	ND[2]	ND
14	Low fat	4.29 ± 0.00[c]	5.12 ± 2.55[a]	5.63 ± 0.11[d]	ND
	Whole fat	3.76 ± 0.01[b]	ND	ND	ND

[a–d]Values with different superscripts within the same column were statistically significant ($P < 0.05$).

[1]Means ± standard error of the means.

[2]Not detectable.

Table 2. Correlation coefficient (R^2) and mean square error (MSE) values of neurons in the topology optimization

Neurons in hidden layer	MSE	R^2
1	2.58608	0.8928
2	3.26711	0.6633
3	2.83637	0.997
4	3.88688	0.8994
5	1.41049	0.999
6	1.19408	0.935
7	4.58721	0.8912
8	6.54816	0.9214
9	7.59667	0.6281
10	4.55557	0.8185
11	1.13805	0.8086
12	1.30613	0.9739
13	1.73334	0.8952
14	1.01199	0.992
15	1.63529	0.6819
16	3.75031	0.4629
17	1.09603	0.8422
18	1.63529	0.7877
19	1.83138	0.1869
20	1.15206	0.9569

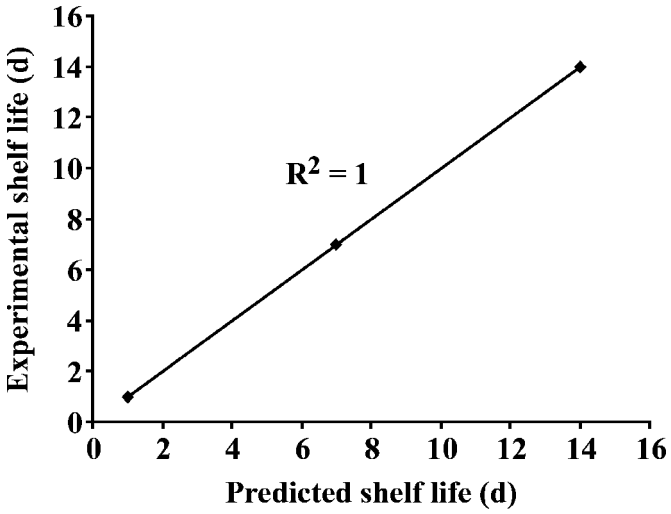

Figure 5. Correlation of experimental vs. neural network values of time with training data set using the optimal network, with 1 hidden layer, 5 neurons per hidden layer, and a training data set of 22 cases.

training of the ANN. Moreover, the final use of the ANN must be considered to select the adequate ANN topology. It is necessary to take into account that light topologies (fewer neurons) involve smaller learning data sets than heavy topologies (more neurons). The learning step is therefore easier and faster and the final ANN calculation time is also shorter. Twenty configurations of ANN topology were conducted (Table 2). As seen from Table 2, configurations 14, 3, and 5 resulted in correlation coefficients (R^2) of 0.992, 0.997, and 0.999, respectively. Because the correlation coefficient of configuration 5 was closest to 1, it was selected as the optimal ANN topology to give the best prediction.

The performance of the ANN model for the training and validation data sets is presented in Figures 5 and 6, respectively. These figures show a comparison of model predictions with the experimental values of shelf life. The results show that the training procedure for shelf-life prediction was very successful and that a perfect match was obtained between the measured and the calculated values. The R^2 values of the ANN model were 1 for the training set and 0.9996 for the testing/validation set.

The performance of ANN models was clearly superior for training the data sets and was reasonably good for testing the data sets. The ANN predictions for testing data sets can be considered to be good predictions, because the ANN predicted values for unseen data sets, whereas regression equations of the statistical models in McClure et al. (1994) and Zaika et al. (1994) were developed for the complete data sets (i.e., all data sets were used for developing the statistical models). Unless the number of data points is much larger than the number of parameters in the polynomial regression equations, there is no assurance that predictions for unseen data would be accurate (Specht, 1991; Barayani and Roberts, 1995). Bratchell et al. (1990) examined how the quantity of data sets can affect model predictions and reported that the response surfaces produced by the model developed from partial data sets were quite different from those produced by the model developed

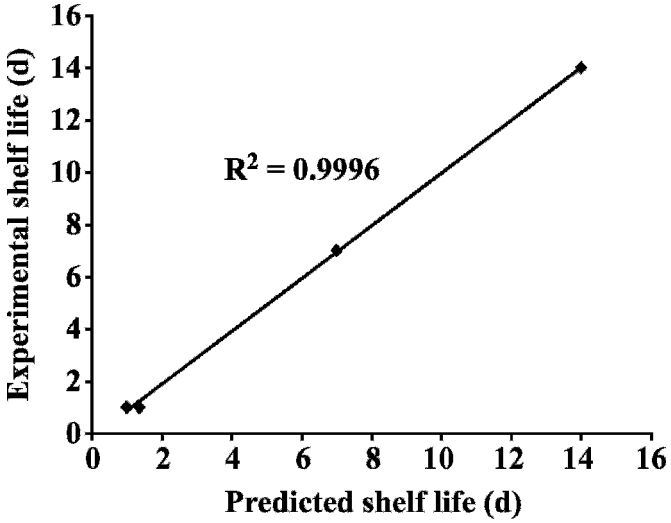

Figure 6. Correlation of experimental vs. neural network values of time with validation data set using the optimal network, with 1 hidden layer, 5 neurons per hidden layer, and a validation data set of 8 cases.

from complete data sets. Therefore, the predictions of statistical models may not be accurate for unseen data.

The simplified algebraic equations derived from the ANN model for the prediction of shelf life with pH, microbial counts, and color analysis are presented in Appendix A. With a priori knowledge of a yogurt's pH, microbial counts, and color analysis, control of its expiration date and prediction of shelf life can be computed using these equations compiled in a simple subroutine.

CONCLUSIONS

Combining neural networks with objective readings can result in powerful quality predictions. The modeling results showed that there was excellent agreement between the experimental data and predicted values, with a high determination coefficient (R^2 = 0.9996) showing that the developed model was able to analyze nonlinear multivariant data with very good performance, fewer parameters, and shorter calculation time. Although ANN has been used in many applications in the agri-food industry, this is the first study modeling the prediction of shelf life of yogurt using ANN. The use of ANN provides an inexpensive and easy technique for evaluation of yogurt quality parameters.

The conceptual ANN model provides a database and an alternative generic framework for the modeling of shelf life of yogurt. This model has potential to be used as an alternative method to control the expiration date, estimate the shelf life of yogurt, and ensure the safety of the product.

ACKNOWLEDGMENTS

We thank Necla Demir for granting permission to use her equipment and facility and we acknowledge her technical guidance. This research was supported by Süleyman Demirel University, Research Foundation (Project No: 0947-YL-04).

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APPENDIX

Equations defining E1 to E6 and F1 to F5 are the summation and activation functions of each neuron, respectively, where c1 to c12 represent color values obtained from the machine vision system.

$$\begin{aligned}E1 = {} & (\text{pH} \times 0.0617659) + (\text{TCA} \times 2.8447) \\ & + (\text{YMC} \times -3.3153) + (\text{CC} \times 0.266086) \\ & + (\text{c1} \times -6.5044) + (\text{c2} \times -2.7020) + (\text{c3} \times 0.2297) \\ & + (\text{c4} \times 0.2047) + (\text{c5} \times 0.0358) \\ & + (\text{c6} \times -0.001795) + (\text{c7} \times -0.0189) \\ & + (\text{c8} \times -0.017515) + (\text{c9} \times 0.68225) + (\text{c10} \times -0.1956) \\ & + (\text{c11} \times -0.7215) + (\text{c12} \times 4.1088) - 1.2609\text{e}^{-005}\end{aligned} \quad [A1]$$

$$F1 = \frac{1}{1 + e^{-E1}} \quad [A2]$$

$$\begin{aligned}E2 = {} & (\text{pH} \times 0.125175) + (\text{TCA} \times 1.3841) \\ & + (\text{YMC} \times -25.3261) + (\text{CC} \times -2.096573) \\ & + (\text{c1} \times -5.6961) + (\text{c2} \times 2.6693) + (\text{c3} \times 0.995) \\ & + (\text{c4} \times 0.6039) + (\text{c5} \times -0.04908) \\ & + (\text{c6} \times -0.05308) + (\text{c7} \times -0.0488) \\ & + (\text{c8} \times -1.6521) + (\text{c9} \times -6.0447) + (\text{c10} \times -0.267) \\ & + (\text{c11} \times -1.7577) + (\text{c12} \times -8.02148)\ 3.3044\end{aligned} \quad [A3]$$

$$F2 = \frac{1}{1 + e^{-E2}} \quad [A4]$$

$$\begin{aligned}E3 = {} & (\text{pH} \times -10.9863) + (\text{TCA} \times -4.8707) \\ & + (\text{YMC} \times 2.5082) + (\text{CC} \times 0.0922) + (\text{c1} \times -10.3972) \\ & + (\text{c2} \times -2.7132) + (\text{c3} \times -4.08124) \\ & + (\text{c4} \times -0.6766) + (\text{c5} \times -0.7825) \\ & + (\text{c6} \times -0.2004) + (\text{c7} \times -0.3464) + (\text{c8} \times 0.5861) \\ & + (\text{c9} \times 18.9605) + (\text{c10} \times 13.5945) + (\text{c11} \times 1.0864) \\ & + (\text{c12} \times -15.69526) - 2.5068\end{aligned} \quad [A5]$$

$$F3 = \frac{1}{1 + e^{-E3}} \quad [A6]$$

$$\begin{aligned}E4 = {} & (\text{pH} \times -0.017812045) + (\text{TCA} \times 0.13228) \\ & + (\text{YMC} \times -3.371) + (\text{CC} \times -0.10783) + (\text{c1} \times -8.6644) \\ & + (\text{c2} \times -2.4376) + (\text{c3} \times -0.11898) \\ & + (\text{c4} \times -0.04318) + (\text{c5} \times -0.0820) \\ & + (\text{c6} \times -0.05122) + (\text{c7} \times 0.00975) + (\text{c8} \times -0.14686) \\ & + (\text{c9} \times 7.0256) + (\text{c10} \times 5.03388) + (\text{c11} \times 0.4698) \\ & + (\text{c12} \times 7.18328)\ 7.0396\end{aligned} \quad [A7]$$

$$F4 = \frac{1}{1 + e^{-E4}} \quad [A8]$$

$$\begin{aligned}E5 = {} & (\text{pH} \times 0.31354318) + (\text{TCA} \times 30.0183) \\ & + (\text{YMC} \times 7.1545) + (\text{CC} \times 1.977713) + (\text{c1} \times 2.6794) \\ & + (\text{c2} \times -3.3225) + (\text{c3} \times 0.13549) \\ & + (\text{c4} \times -0.0083) + (\text{c5} \times 0.5969) \\ & + (\text{c6} \times 0.12248) + (\text{c7} \times 0.73401) + (\text{c8} \times 0.7373) \\ & + (\text{c9} \times -4.5281) + (\text{c10} \times -1.49695) + (\text{c11} \times 1.2050) \\ & + (\text{c12} \times 13.03948)\ 2.7726\end{aligned} \quad [A9]$$

$$F5 = \frac{1}{1 + e^{-E5}} \quad [A10]$$

$$\begin{aligned}E6 = {} & (\text{F1} \times 14.4456) + (\text{F2} \times -69.2291) \\ & + (\text{F3} \times -16.3589) + (\text{F4} \times -27.2027) \\ & + (\text{F5} \times -30.7632) - 0.13352\end{aligned} \quad [A11]$$

$$Days\ (D)/15 = \frac{1}{1 + e^{-E6}} \quad [A12]$$