Monitoring the authenticity of low-fat yogurts by an artificial neural network

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ABSTRACT

The growing consumption of low- and reduced-fat dairy products demands routine control of their authenticity by health agencies. The usual analyses of fat in dairy products are very simple laboratory methods; however, they require manipulation and use of reagents of a corrosive nature, such as sulfuric acid, to break the chemical bounds between fat and proteins. Additionally, they generate chemical residues that require an appropriate destination. In this work, the use of an artificial neural network based on simple instrumental analyses, such as pH, color, and hardness (inputs) is proposed for the classification of commercial yogurts in the low- and reduced-fat categories (outputs). A total of 108 strawberry-flavored yogurts (48 probiotic low-fat, 36 low-fat, and 24 full-fat yogurts) belonging to several commercial brands and from different batches were used in this research. The statistical analysis showed different features for each yogurt category; thus, a database was built and a neural model was trained with the Levenberg-Marquardt algorithm by using the neural network toolbox of the software MATLAB 7.0.1. Validation with unseen data pairs showed that the proposed model was 100% efficient. Because the instrumental analyses do not require any sample preparation and do not produce any chemical residues, the proposed procedure is a fast and interesting approach to monitoring the authenticity of these products.

Key words: artificial neural network, low-fat yogurt, reduced-fat yogurt, quality control

INTRODUCTION

Nowadays, growing public concern regarding caloric and fat intake has led to worldwide trends toward the consumption of low- or reduced-fat foods (Oliveira and Assumpção, 2000). Yearly, more than 180 low- and reduced-fat foods are launched in the Brazilian market,

achieving a growth rate of 870% in the last decade (Fadini, 2005). The yogurt market constitutes a segment with great potential for expansion in Brazil. In 2005, it reached nearly 482.4 thousand tonnes, representing an increase of 10.9% over the previous year and 42.6% over 2001. In monetary value, the records are even greater: the \$2.03 billion amount achieved in 2005 corresponds to a 21.3% growth over 2004 and 99% when compared with 4 yr previously. Per capita consumption of the product in Brazil was 3.062 kg in 2005, compared with 2.311 kg in 2001 (Bourroul, 2006). Although there are no exact figures on the participation of low-fat yogurt in this market, it is known to represent a significant contribution to these numbers.

An artificial neural network (ANN) is a powerful computational technique for correlating data by using a large number of simple processing elements. Its main advantage over conventional modeling is that it can handle complex nonlinear relationships with ease, even when the exact nature of such behavior is unclear. In this context, the ANN is well suited for food quality prediction, which is a complex task because of the nature of the interrelationships among various quality parameters and processing conditions (Ni and Gunasekaran, 1998). Huang et al. (2007) described the following potential applications of ANN in food science and technology: modeling microbial growth to predict food safety, and, from spectroscopic data, predicting the physical, chemical, and functional properties of food products during processing and distribution.

Artificial neural networks are used more as a mathematical model than as a biological one. It can be thought of as a way of modeling a functional relationship between a set of inputs and a set of corresponding output variables (Marini, 2009). In dairy products, ANN have been used successfully 1) to check the authenticity of Ossolano cheese, aimed at identifying its production area, because it is a product with a protected designation of origin (Barile et al., 2006); 2) to discriminate Emmental cheese according to the geographic identification of its production area (Pillonel et al., 2005); 3) to identify bacterial causes of mastitis in dairy herds, to evaluate the adoption of good agricultural practices

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Table 1. Range of nutrients in the yogurts¹

Yogurt	Protein (%)	Fat (%)	Carbohydrates	Stabilizers
Full-fat	1.8 to 3.5	2.0 to 5.4	17 to 27	Gelatin, xanthan gum
Low-fat	4.3 to 7.2	0.9 to 2.2	6.9 to 7.4	Gelatin, xanthan gum
Probiotic low-fat	3.2 to 4.5	0 to 2.5	11 to 19	Gelatin, whey powder, xanthan gum

¹As stated on the label (% = wt/wt %).

(Heald et al., 2005); 4) to determine the protein level in raw milk (Etzion et al., 2004); 5) to predict moisture in cheese during commercial production (Jimenez-Marquez et al., 2005); 6) to model the loss of yogurt quality and discriminate varieties of the product, as well as to predict the storage period (He et al., 2006; Shao et al., 2007; Sofu and Ekinici, 2007); and 7) to detect mastitis pathogens by analyzing volatile bacterial metabolites (Hettinga et al., 2008) and controlling operational parameters of the churning process in continuous butter manufacture (Funahashi and Horiuchi, 2008). Recently, the use of ANN for quantitative determination of the protein content in yogurt has been reported (Khanmohammadi et al., 2009).

The growing consumption of low- and reduced-fat dairy products demands routine control of their authenticity by health agencies. The usual analyses of fat in dairy products (e.g., Roesse-Gottlieb, Schmid-Bondzynski-Ratzlaff, Mojonier, and Gerber) are very simple laboratory methods; however, they require the manipulation and use of reagents of a corrosive nature, such as sulfuric acid, to break the chemical bonds between fat and proteins. Additionally, they generate chemical residues that require an appropriate destination.

In this work, the use of an ANN based on simple and fast instrumental analyses, such as pH, color, and hardness, is proposed for the classification of commercial yogurts into low-fat, probiotic low-fat, and full-fat product categories, thus providing an additional tool for quality control monitoring of these products.

MATERIALS AND METHODS

Sampling

A total of 108 strawberry-flavored set yogurts (48 probiotic low-fat, 36 low-fat, and 24 full-fat yogurts) belonging to 12 commercial brands and from different batches were used in this research. The composition of the products was obtained by visual inspection of their labels, as reported in Table 1. Five samples of each commercial brand were purchased randomly from several supermarkets in the city of Campinas, São Paulo, Brazil, and kept under refrigeration (3 to 5°C). The commercial brands analyzed make up 100% of the Brazilian yogurt market.

pH and Color

The pH of yogurt samples was determined by means of a digital potentiometer, in quintuplicate, by direct immersion of the electrode (Tecnal, São Paulo, Brazil) into the sample (20 to 25 mL) at room temperature.

The instrumental color of each sample was measured in quintuplicate by using a Hunter Colorimeter, Spectrocolor Color System Model 96 (BYK Gardner, Silver Spring, MD). Samples were added to optical glass cells 3.8 cm high and 6 cm in diameter. Reflection spectra and also the CIELAB color parameters for illuminant D65 and 10° vision angle were registered: L (brightness), a (red component), and b (yellow component).

Hardness

Hardness was determined using a universal TAXT2 Texture Analyzer (Stable Micro Systems, Haslemere, UK). Individual refrigerated yogurts cups, each containing 200 mL of sample, were analyzed immediately inside the container in 5 repetitions after withdrawal from refrigerated storage, taking 3 results with minor deviations. A double compression test was performed using a 2.50-cm-diameter acrylic cylinder in the computer-controlled texture analyzer. The distance traveled by the cylinder in the sample was 10 mm at a speed of 3 mm/s. The hardness attribute was determined according to the recommendations of Rawson and Marshall (1997), which correspond to the time of the first peak of the double compression curve.

Statistical Analysis

The results of the instrumental analyses were submitted to statistical analysis by one-way ANOVA (*F*-test). The average values were compared with the Tukey test, at a 5% level of significance, using the Statistica Program for Windows, version 7.0 (StatSoft Inc., Tulsa, OK).

ANN Modeling

Theory. Artificial neural networks are mathematical models composed of several neurons, arranged in different layers (input, hidden, and output) intercon-

Figure 1. Neural network structure.

nected through a complex network. The multilayer feed forward was used, which is the most popular of the many architectures currently available (Figure 1). According to equation [1], a neuron is responsible for the summation of all signals from the previous layer's neurons, y_j (amplified or weakened by weight values, $w_{k,j}$), and a value called bias (b_k). A transfer function, f , such as a hyperbolic tangent, logarithmic sigmoid, or linear function, is used for the activation of the neuron output, y_k :

$$y_k = f \left[\sum_{j=1}^N (w_{k,j} y_j) + b_k \right]. \quad [1]$$

Data processing within the ANN structure is executed collectively and simultaneously through the dense network of neurons and their connections.

Training the ANN. Once the network weights and biases have been initialized, the network is ready for training. The training process requires a set of examples of proper network behavior—network inputs and target outputs. During training, the weights and biases of the network are iteratively adjusted to minimize the net-

work objective function. The basic training algorithm is the backpropagation algorithm, in which the weights are moved in the direction of the negative gradient (Demuth et al., 2002).

A successful method for improving generalization is called regularization. This involves modifying the objective function, which is normally chosen to be the sum of squares of the network errors on the training set. It is possible to improve the generalization if one modifies the objective function by adding a term that consists of the mean of the sum of squares of the network weights and biases:

$$F = \beta \cdot SSE + \alpha SSW, \quad [2]$$

where SSE is the sum of squared errors, SSW is the sum of squares of the network weights, and β and α are objective function parameters (Demuth et al., 2002). According to Foresee and Hagan (1997), using this objective function will cause the network to have smaller weights and biases, and this will force the network response to be smoother and less likely to overfit.

Neural network training can be made more efficient if certain preprocessing steps are performed on the

Table 2. Instrumental analyses of yogurts

Item ¹	Full-fat	Low-fat	Probiotic low-fat
pH	4.15 ^a	4.18 ^a	4.02 ^b
Hardness (N)	77.50 ^a	73.82 ^a	60.83 ^b
L	70.05 ^a	69.02 ^a	67.06 ^b
a	13.94 ^a	13.89 ^a	18.10 ^b
b	2.65 ^a	2.80 ^a	2.72 ^a

^{a,b}Values in the same row followed by the same superscript letter do not differ significantly according to the Tukey test ($P < 0.05$).

¹L = brightness; a = red component; b = yellow component.

network inputs and targets. Thus, before training the network the training data were normalized in the range $[-1,1]$, as follows:

$$x_n = 2 \frac{(x - x_{\min})}{(x_{\max} - x_{\min})} - 1, \quad [3]$$

where x_n is the normalized value for the variable, and $x_{\min}$ and $x_{\max}$ are the minimum and maximum of each variable x .

The Levenberg-Marquadt backpropagation algorithm coupled with the regularization method was used for training the neural model (*trainbr* function in MATLAB software; The MathWorks Inc., Natick, MA). The stop criteria was 1,000 epochs or the maximum sum of squared errors equal to 0.0001 during net training.

Model for Monitoring the Category of Yogurt

The category of yogurt, in terms of its fat content, can be determined by the neural model from instrumental analysis data, as shown in Figure 1. When the network output finds number 1, the category is probiotic low-fat yogurt; when it finds number 2, the category is full-fat yogurt; and when it finds number 3, the category is low-fat yogurt.

A feed-forward architecture network containing 2 hidden layers was used. In this study, because the output was not a continuous function, the usual simple structure of one hidden layer was not properly applied. The transfer functions hyperbolic tangent and linear were used in the hidden and output layers, respectively.

Performance Criteria

The best network structure selection was performed by comparing ANN-predicted data (y^{pred}) with the actual category of yogurt (y^{actual}). The root mean square deviation (**RMSD**) was evaluated according to equation [4] and by visual graphical deviation analysis as well:

$$RMSD = \sqrt{\frac{\sum_{j=1}^n (y_j^{pred} - y_j^{actual})^2}{n}}, \quad [4]$$

where n is the number of data points in the test data set.

RESULTS AND DISCUSSION

Table 2 shows the average values obtained from the instrumental analyses of the yogurts. Except for the b parameter, probiotic low-fat yogurts showed significant differences for all the parameters ($P < 0.05$) compared with full-fat and low-fat yogurts, respectively.

The pH range found (4.02 to 4.15) was related to the different lactic cultures used by the manufacturers, each with distinct lactose consumption features and acidification capacities. In addition, the control of the manufacturing process (with some manufacturers interrupting the fermentation process when pH reaches between 4.8 and 4.5) could justify the results obtained. Batista et al. (2008) found similar results when evaluating the physicochemical parameters of commercial yogurts and fermented milks to evaluate their possible use for lactose-intolerant people. According to Tamime and Robinson (2000), a pH value between 3.7 and 4.6 is normally found for fermented milks. The ideal values are between 4.0 and 4.4, because bitter and acid sensations are not present at this interval. All the samples fit in this range.

Full-fat and low-fat yogurts also presented greater hardness values than probiotic low-fat yogurts ($P < 0.05$), which may be related to the usual homogenization present in yogurt processing. During this operation, there is a reduction in the size of fat globules and the fat surface area increases dramatically because the surface of homogenized fat particles can interact with the protein matrix of acid gels; this results in reinforcement of the gel strength because of interactions between the fat globule membrane and the protein matrix (Sodini et al., 2004). Therefore, there is an increase in hardness values. Another factor is the amounts of stabilizers used in the formulation of the probiotic low-fat products. In spite of several works indicating that addition of xanthan gum (El-Sayed et al., 2002; Soukoulis et al., 2007; Teles and Flores, 2007) and gelatin (Fiszman et al., 1999) can produce a yogurt with improved texture, preventing the wheying-off defect, there is increasing demand for natural products that use fewer or no additives or stabilizers. Therefore, is it probable that only a few of these compounds are being incorporated to reinforce the positive health image of these products. This finding is important because the fortification of

Figure 2. Target (actual) and neural model prediction of the yogurt categories for the test set. Categories: 1) probiotic low-fat yogurt; 2) full-fat yogurt; and 3) low-fat yogurt.

milk with increasing amounts of whey power has a detrimental impact on the gel consistency because of the more open structure formed with a low CN content, which makes the aggregate network more sensitive to syneresis (Gonzalez-Martinez et al., 2002).

In fact, whey or serum separation, which is also called wheying-off, is a common defect during storage of fermented milk products such as yogurts, and can be noticed by the appearance of whey on the surface of a gel. Manufacturers try to prevent it by increasing the TS content of milk, by subjecting the milk to a severe heat treatment, or by adding stabilizers (Lucey and Singh, 1998; Lucey, 2002). High incubation temperatures and low starter inoculum levels adopted during yogurt processing are perceived as the main factors involved in this technological defect; the former makes the gel network more prone to rearrangements, whereas the latter results in a slower acidification, providing more time for dissolution of colloidal calcium phosphate, especially during the early stages of gelation (Lee and Lucey, 2004b). Salvador and Fiszman (2004) reported lower hardness values for whole-milk yogurt compared with skim-milk yogurt under different storage temperatures,

with there being a significant effect for different storage temperatures. The authors attributed this finding to the lower fat and higher protein contents.

It is important that most of the labels presented a mixture of stabilizers in a qualitative approach. Thus, it was very difficult to determine the exact amount of these additives used.

The lower hardness values for probiotic yogurts suggest a small contribution of this microbial group for this parameter. In fact, probiotic bacteria present slow growth in milk because of a lack of proteolytic activity (Shah, 2000), and are used only as adjunct cultures. The development of fermented milks supplemented with probiotic bacteria covers ingredient supplementation such as cysteine, whey protein concentrate, acid CN hydrolysates, or tryptone (Dave and Shah, 1998; Antunes et al., 2005). Oliveira and Damin (2003) also reported different results in evaluating the hardness of fermented milk supplemented with probiotic cultures. They presented lower values for fermented milks without this microbial group, which can be related to the different strains used and their behavior during the fermentation step, added to their capacity to produce

Figure 3. Deviation from the target points of the test set.

exopolysaccharide (**EPS**), which contributes to an improvement in yogurt texture (Sodini et al., 2004). The use of EPS-producing bacteria is a viable alternative to improve texture and viscosity in yogurts because, in some countries, the addition of stabilizers in this food are not permitted; therefore, they can reduce the demand for additives (Rana and Gandhi, 2004). The use of EPS-producing starter cultures decreases firmness and syneresis and changes the protein matrix in yogurts produced with a higher level of TS, although the absence of differences was noted between samples using capsular EPS- and ropy EPS-producing starter cultures (Amatayakul et al., 2006).

Ranges in the color instrumental parameters (L: 67.06 to 70.05; a: 13.89 to 18.10, where L refers to brightness

and a refers to redness) are related, in the latter one, to different levels of fruit pulp added to the yogurt formulations and the homogenization of milk destined for the manufacture of yogurts. The fruit preparations for addition to fermented milks are specially designed to meet marketing requirements, and are formulated to furnish adequate viscosity for thorough blending without significant dilution of the finished product (O'Rell and Chandan, 2006). In general, the level Brazilian dairy producers add to fruit pulp ranges from 5 to 15% (wt/vol).

These different features presented by each yogurt category allow for the suitable design of a neural model to check the authenticity of a low-fat commercial yogurt. With the results of these analyses, a database was built

Table 3. Weights between neurons at input (I) and the first hidden (H1) layers

Neuron	I ₁	I ₂	I ₃	I ₄	I ₅
H1 ₁	−1.5491	−3.3504	0.8691	−2.7527	2.2716
H1 ₂	−1.7231	−3.7110	3.2127	−2.3623	2.7486
H1 ₃	−1.6934	−0.4114	−1.0550	1.0980	2.5646
H1 ₄	0.1954	−0.0597	1.6071	1.3264	−2.5640

Table 4. Weights between neurons at the first (H1) and second hidden (H2) layers

Neuron	H1 ₁	H1 ₂	H1 ₃	H1 ₄
H2 ₁	2.7785	−3.2356	0.7604	−0.7306
H2 ₂	1.8773	−1.5554	3.1797	3.2699
H2 ₃	0.4129	0.2165	−2.0547	−4.1194
H2 ₄	2.6129	−1.7709	2.1874	−1.0307

and a neural model was trained based on it by using the neural network toolbox of the software MATLAB 7.0.1 (The MathWorks Inc.).

Artificial Neural Modeling

According to a well-known heuristic rule, for the ANN validation, a set of pairs representing 25% of the whole data set is required. From 108 experimental pairs of data, 27 were taken for the validation test of the model. Taking into account the different numbers of samples for each yogurt category, the data set was made up of 12 data pairs of category 1 (probiotic low-fat yogurt), 6 data pairs of category 2 (full-fat yogurt), and 9 pairs of category 3 (low-fat yogurt).

From trial-and-error methodology, the best number of hidden neurons was found: 4 nodes for each hidden layer. With only 296 epochs, the training procedure found a sum of squared errors of 0.045 for the 81 training pairs. The training data set RMSD found was 0.024.

Figure 2 shows a visual comparison between the neural model prediction and the actual target categories. The accuracy of the neural model predictions for categories 1 and 3 was clear; however, for category 2 the performance of the model decreased.

Figure 3 shows graphically the deviation from the target points. The performance criteria obtained for the test set were as follows: a maximum deviation of 0.189 (category 2, point number 16) and an RMSD 0.063. This means that the maximum deviation reached approximately 10% of the category value. However, if one considers that only the real integer number presents some meaning (yogurt category), the prediction number might be rounded to an integer, and this integer number would certainly be the correct target. Prediction mistakes would appear only if deviations reached 50%. From this analysis, it could be concluded that the neural model presented 100% correct predictions.

Table 5. Weights between neurons at the second hidden (H2) and output (O) layers

Neuron	H2 ₁	H2 ₂	H2 ₃	H2 ₄
O	−2.2413	3.2500	2.2484	−2.1549

Table 6. Biases found for the hidden layers and for the output neuron (O)

Neuron layer	1	2	3	4
H1	−1.9059	−2.7048	−0.9361	0.7706
H2	−4.7455	−0.6702	0.4470	2.1818
O	−0.0914	—	—	—

It is relevant remember that although the ANN modeling showed adequate classification of the yogurts according their category, its application is limited to set yogurts having the nutrient composition shown in Table 1, in either a qualitative or a quantitative approach. Because several possibilities exist for the use of different ingredients and technological parameters in the yogurt industry, any product that has different ingredients in its formulation can be included in the model if an adequate number of samples are analyzed.

To release this model to readers, the weights and biases obtained from training are shown in the Appendix. Before using this model, normalization of the variables is required according to equation [3], and the valid region of use should be checked as well.

CONCLUSIONS

From the cited instrumental analyses, full-fat yogurts showed significant differences compared with probiotic low-fat and low-fat yogurts. Probiotic yogurts, independent of the fat level, presented higher hardness values. With the results of pH, hardness, and color analysis of samples from the 3 categories of yogurt, a database was built and a neural model was trained based on it.

Because the problem is discrete (not continuous), one hidden layer was not sufficient for the neural approach. Thus, 2 hidden layers were used, in which hyperbolic tangent functions were applied as activation functions. Four neurons were used, and each had a hidden layer. A linear function was used in the output layer. Because the trained neural model is made up of algebraic equations, it could be implemented easily in an electronic worksheet.

Validation with unseen data pairs showed that the proposed model was 100% efficient in predicting the yogurt category. Because the instrumental analyses do not require any sample preparation and do not produce any chemical residues, the proposed procedure was shown to be a fast and interesting approach to monitoring the authenticity of these products.

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APPENDIX

The optimal neural model parameters (weights and biases) are summarized in Tables 3 to 6. The neural model structure used was 5-4-4-1 (I-H1-H2-O). The input variables must follow the sequence shown in Table 1. Normalization of variables is required according to equation [3]. This model is valid for the following input ranges: pH [3.72, 4.68], hardness [43.52, 168.89], L [62.53, 76.99], a [10.47, 24.75], and b [0.38, 5.09].