Available online at www.sciencedirect.com
ScienceDirect
Sensors and Actuators B 119 (2006) 64–69
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Electronic sensor array coupled with artificial neural network for detection of *Salmonella* Typhimurium

Ubonrat Siripatrawan [a,*], John E. Linz [b], Bruce R. Harte [c]

[a] *Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[b] *Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824-1314, USA*
[c] *School of Packaging, Michigan State University, East Lansing, MI 48824-1314, USA*

Received 2 November 2005; received in revised form 24 November 2005; accepted 26 November 2005
Available online 18 January 2006

Abstract

An electronic sensor array with 12 non-specific metal oxide sensors was evaluated for its ability to monitor volatile compounds in super broth, alone and in super broth inoculated with *Salmonella* Typhimurium at 37 °C for 2–14 h. Using discriminant function analysis (DFA), it was possible to differentiate super broth alone from that containing *Salmonella* Typhimurium. The potential to predict the number of *Salmonella* Typhimurium was investigated using an artificial neural network (ANN). The ANN was comprised of an input layer, one hidden layer and an output layer, with a hyperbolic tangent sigmoidal transfer function in the hidden layer and a linear transfer function in the output layer. Good prediction was found as measured by a regression coefficient ($R^2 = 0.998$) between actual and predicted data.

Keywords: Electronic sensor array; Artificial neural network; *Salmonella* Typhimurium

1. Introduction

Microbiological safety and quality testing in the food industry have been based upon traditional plate count methods [1]. These conventional techniques are laborious and time consuming. The development of alternative analytical techniques that are rapid and simple has become increasingly important to reduce per-sample-time investment and to conduct real time analyses. For continuous monitoring with fast response times, chemical sensors can be used. This concept has been actualized in an electronic nose or electronic sensor array [2–5].

Microorganisms can be characterized by identification of specific metabolites associated with their activities. However, many metabolites may be common to several microorganism species. Therefore, the differences between samples often relate to a complex balance between pattern of volatiles rather than to a major change in one or two constituents. Electronic nose has the potential to be a sensitive, fast, one-step method to characterize a wide array of different volatile chemicals [6]. However, the electronic nose generated multi-dimensional data that was difficult to handle and visualize. This underlines the important of using multivariate data analysis (MVA) to extract the specific information necessary to target a specific microorganism. Pattern recognition interpretation techniques, such as discriminant factor analysis (DFA) and artificial neural network (ANN), provide complementary information which was simply unachievable by conventional data analysis [7,8].

The objective of this study was to investigate the ability of the electronic sensor array in conjunction with DFA and an ANN to determine the number of *Salmonella* Typhimurium in nutrient media.

2. Methodology

2.1. Preparation of test solution

Super broth consisting of 32 g tryptone, 20 g yeast, 5 g NaCl, and 5 ml of 1N NaOH per liter was used as a basal medium. Before being utilized, *Salmonella* Typhimurium was transferred from the stock culture to super broth and incubated overnight at 37 °C. *Salmonella* Typhimurium (100 CFU/ml) was inoculated into super broth, and 5 ml of super broth were transferred

* Corresponding author. Tel.: +662 2185536; fax: +662 2544314.
E-mail address: ubonratana.s@chula.ac.th (U. Siripatrawan).

0925-4005/$ – see front matter
doi:10.1016/j.snb.2005.11.059

into standard 20 ml headspace vials and sealed with PTFE-lined Teflon caps (Alpha M.O.S., Hillsborough, NJ). The cultures were allowed to grow in vials at 37 °C in a gyrotory shaker (G-25 New Brunswick Scientific Corporation, New Brunswick, NJ) at 100 rpm. Samples were periodically analyzed after incubation at 2, 4, 6, 8, 10, 12, and 14 h at 37 °C by a colony counting and electronic sensor techniques.

2.2. Colony counting method

Samples were serially diluted in sterile Butterfield's phosphate buffer. A series of dilutions was prepared from the stock suspension. Serially diluted samples were plated in duplicate using 3M Petrifilm aerobic count plates (3M Industrial Markets, St. Paul, MN) to determine total bacteria. All plates were incubated at 35 °C for 48 ± 2 h. Plate counts were recorded as CFU/ml.

2.3. Electronic sensor array for monitoring of volatiles

An electronic sensor (Fox 3000, Alpha M.O.S., Hillsborough, NJ) was used for monitoring changes in volatiles produced by *Salmonella* Typhimurium in super broth medium. The volatile analysis system combines a measurement chamber for generating the volatile compounds and a detection system made up of 12 metal oxide sensors (SYLG, SYG, SYAA, SYGH, SYGCTI, SYGCT, T301, P101, P102, P401, T702, and PA2). The descriptors associated with the sensors are shown in Table 1. This instrument was linked to an autosampler capable of analyzing a total of 64 samples. Samples were placed in glass vials and sealed with crimped PTFE/metal septa.

Samples were placed in the HS100 auto-sampler in arbitrary order. Prior to analysis, the vial was removed from the sample tray and placed in a temperature-controlled chamber. The vial temperature was held at 35 °C while being spun in order to produce an equilibrated headspace. The time the vial remains in this chamber is the headspace generation time. The automatic injection unit heats the samples to 35 °C using an incubation time of 300 s. The temperature of the injection syringe was 40 °C. The injector needle then removes 2.5 ml of headspace and injects it into the sensor chamber. The delay time between two injections was 300 s. Each injection was repeated, with separate samples (three times for all variations per day) for 7 days. The electronic signals from the sensors were digitized and then transferred to the control computer. Resistance changes (difference in sensor resistance between air blank and odorous atmosphere) were recorded.

Table 1
Sensor types and volatile descriptors

Sensors	Description of volatile analyses
P101, P102, SYGCT	Non-polar volatiles, methane, propane, hydrogen bonding compounds, aldehydes
PA2, SYAA, T301	Polar compounds, alcohol
T702	Alcohol, aromatic compounds
SYG, SYGH, SYGCTI	Ammonia and ammonia derivatives, sulphur, amines and amine containing compounds
P401, SYLG	Chlorinated compounds, aldehydes

2.4. Data analysis

Data were made up of 120 samples from 8 subgroups (CTR, ST-2, ST-4, ST-6, ST-8, ST-10, ST-12, and ST-14). Each sample was analyzed using 12 metal oxide sensors. The sensor responses of all 120 samples were arranged in a 120×12 matrix. For ANN analysis, the data sets were partitioned into two subsets: a training set (70% of the data), used to adjust the network weights, and a validation set (the remaining 30% of the data), used to validate the model. The performance of the selected network was then assessed by measuring its performance on a third independent set of data (unseen observations) called a test set. First, data patterns of known identity were presented to the ANN as exemplars. Once the ANN was trained, any data pattern could be presented to it and the output analyzed to find the most likely identity of the pattern. The network was trained by comparing predicted *Salmonella* numbers with their known identify. When an optimized network is obtained, its ability to generalize must be tested by presenting it with an unseen independent set of data and comparing predicted data with known identity. All calculations were carried out using MATLAB 5.2 routines written by the authors, and making use of the toolbox provided by Mathworks (Mathworks Inc., Natick, MA).

3. Results and discussion

3.1. Colony counts

The population of *Salmonella* Typhimurium grown in closed vials containing super broth is shown in Fig. 1. The data were from 15 replicates. Prior to 6 h incubation, the growth of *Salmonella* Typhimurium was slow, probably because they were in the lag phase. Between 4 and 10 h, cultures exhibited exponential growth and numbers increased rapidly from 10^2 to 10^{10} CFU/ml.

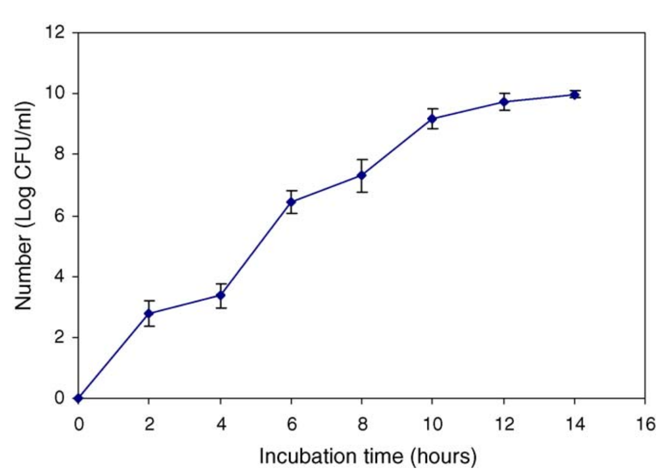

Fig. 1. Growth of *Salmonella* Typhimurium in super broth incubated at 35 °C.

3.2. Electronic sensor array

The data matrix comprised 120 samples from 8 subgroups (CTR, ST-2, ST-4, ST-6, ST-8, ST-10, ST-12, and ST-14) as analyzed using the 12 sensors (SYLG, SYG, SYAA, SYGH, SYGCTI, SYGCT, T301, P101, P102, P401, T702, and PA2). The patterns of the samples difference due to the different pattern of volatile metabolites generated by *Salmonella* Typhimurium produced during the incubation period. In this study, 12 metal oxide sensors were used. Different sensor signal intensities between samples are important to discriminate between samples. Each sensor element changes in resistance (R_{max}) when exposed to volatile compounds. The information from electronic sensor array analysis was extracted from the series of sensor resistances. In order to produce consistent data for the classification, the sensor response was presented with a volatile chemical relative to the base resistance in air, which is the maximum change in the sensor's electrical resistance divided by the initial resistance, as follows:

$$\text{relative resistance change} = \frac{\Delta R_{max}}{R_0} \quad (1)$$

where $\Delta R_{max} = R_{max} - R_0$ = maximum change in the sensor's electrical resistance and R_0 = initial baseline resistance of the sensor. The baseline of the sensors was acquired in a synthetic air saturated steam water at fixed temperature. The $\frac{\Delta R_{max}}{R_0}$ value was used for data evaluation because it gives the most stable result, and is more robust against sensor baseline variation.

Fig. 2 shows the responses of all samples in 8 subgroups to the 12 metal oxide sensors. The sensor array has the potential to be a sensitive, fast, one-step method to characterize volatile compounds in a sample. However, the electronic sensor array generated multi-dimensional data that was difficult to handle and visualize. Therefore, DFA was used as pattern recognition technique to discriminate between different sample groups based on voltametric responses.

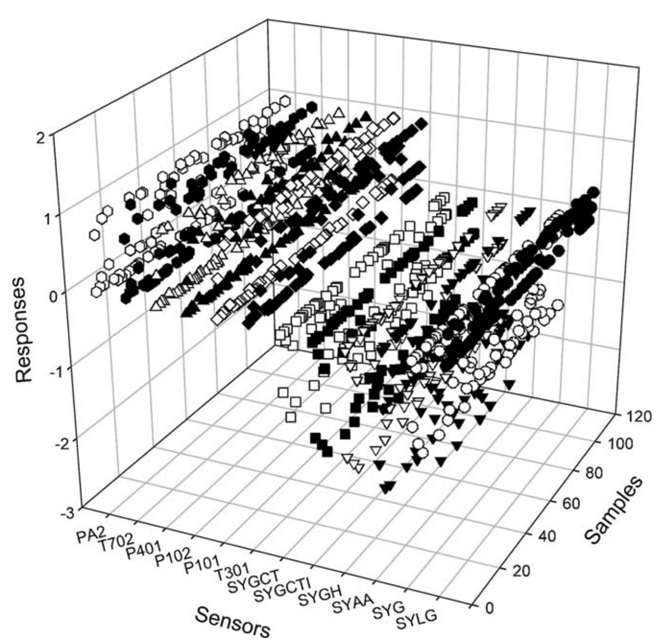

Fig. 2. Electronic sensor responses of control and nutrient media inoculated with *Salmonella* Typhimurium and incubated for 2, 4, 6, 8, 10, 12, and 14 h.

Table 2
Canonical DFA analysis of electronic sensor responses

Discriminant functions	Eigenvalues	Cumulative dispersion (%)
1	382.900	84.70
2	14.432	97.90
3	3.551	99.20
4	0.974	99.90
5	0.107	100.00
6	0.018	100.00
7	0.001	100.00

3.3. DFA

DFA was used to determine whether it is possible to separate two or more individual groups, given measurements for these individuals from several variables. DFA is a method that differentiates between the within- and between-class scatters to derive class-specific feature spaces. For discriminant factors, the variables are chosen according to the characteristics that differing between the groups. These variables are then linearly combined and weighted so that the groups are forced to be as statistically distinct as possible by choosing the linear combination of variates that maximizes the one-way analysis of variance *F*-test, which tests the equality of the means for the linear combinations [8,9].

Typically for DFA if there are *n* variables, $n-1$ discriminant factors were derived [10]. In this study there were eight sample groups. Seven factors were extracted, which accounted for 100% of the total variance in the data set. The results are given in Table 2. Eigenvalues are indicative of the relative importance of the discriminant factor in determining group separation. Although some information was available in later factors, the first two factors had the highest eigenvalue and account for 97.90% of the cumulative proportion of the total dispersion,

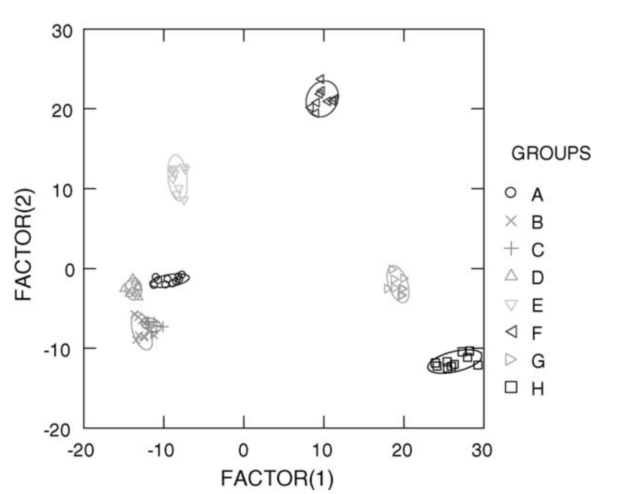

Fig. 3. Classification of samples using DFA. The samples are labeled using the following scheme: control (A), after incubated for 2 h (B), 4 h (C), 6 h (D), 8 h (E), 10 h (F), 12 h (G), and 14 h (H).

Table 3
Coefficients of canonical discriminant factor

Sensors	Canonical discriminant factor						
	1	2	3	4	5	6	7
SYLG	4.55	2.89	11.04	17.69	18.39	14.13	26.77
SYG	−5.00	−0.43	−3.86	2.34	4.59	1.50	3.16
SYAA	6.71	−2.36	−2.24	2.10	0.83	0.79	3.21
SYGH	−2.25	−1.41	−1.00	4.43	−0.35	0.76	−1.28
SYGCTI	1.41	7.35	1.64	−4.20	−13.53	−3.82	2.63
SYGCT	10.08	−5.77	−9.13	4.14	−5.02	5.73	8.13
T301	3.17	−10.14	−1.70	4.93	−11.17	−14.16	−1.04
P101	−6.20	−9.62	−12.50	−10.05	2.27	9.16	7.78
P102	−6.19	27.79	−0.54	12.74	−3.13	−3.55	9.90
P401	−13.73	7.11	−13.54	4.93	1.95	−2.74	0.65
T702	−2.11	−4.59	1.70	−5.02	−13.99	−8.34	2.67
PA2	−8.26	−12.15	−0.10	1.72	−7.10	18.39	−6.33

thus, the data could be displayed using the first two factors (Fig. 3).

Table 3 lists the coefficients of each canonical discriminant factor. The first canonical variable (Factor 1) was the linear combination of the original variables (Eq. (2)) that best discriminate among the groups; the second canonical variable (Factor 2) was orthogonal to the first and was the next best combination of variables (Eq. (3)).

$$\begin{aligned}\text{Factor 1} = {} & 4.55\text{SYLG} - 5.00\text{SYG} + 6.70\text{SYAA} - 2.25\text{SYGH} \\ & + 1.41\text{SYGCTI} + 10.08\text{SYGCT} + 3.17\text{T301} \\ & - 6.20\text{P101} - 6.19\text{P102} - 13.72\text{P401} - 2.11\text{T702} \\ & - 8.26\text{PA2}\end{aligned} \quad (2)$$

$$\begin{aligned}\text{Factor 2} = {} & 2.89\text{SYLG} - 0.43\text{SYG} - 2.36\text{SYAA} - 1.41\text{SYGH} \\ & + 7.35\text{SYGCTI} - 5.77\text{SYGCT} - 10.14\text{T301} \\ & - 9.62\text{P101} + 27.79\text{P102} + 7.11\text{P401} - 4.59\text{T702} \\ & - 12.15\ \text{PA2}\end{aligned} \quad (3)$$

The first canonical variable was then plotted versus the second canonical variable for visual inspection of the data. Sample factor scores were plotted on a DFA graph, and the separation of class-labeled samples is shown in Fig. 3. Classification was performed by assigning a pattern vector to the class with the closest Mahalanobis distance metric [11], as given by

$$D_c^2 = (\mathbf{x} - \boldsymbol{\mu}_c)S(\mathbf{x} - \boldsymbol{\mu}_c)^{\mathrm{T}} \quad (4)$$

where D_c^2 is a squared Mahalanobis distance between two data points, $\mathbf{x}$ the pattern vector being classified, $\boldsymbol{\mu}_c$ the vector of means for subgroup c (c = 1, . . ., 8), T the transformed vector, and S is the pooled variance-covariance matrix. The samples (CTR, ST-2, ST-4, ST-6, ST-8, ST-10, ST-12, and ST-14) were attributed to the groups whose average Mahalanobis distance was similar to the average value of the data points of a certain group, using the real qualitative group samples from the first day of inoculation, the control on the first day of preparation and after incubated for 1 day overlapped.

From the DFA pattern, the data were classified into seven groups. The samples inoculated with *Salmonella* Typhimurium and incubated for 2 and 4 h overlapped. These sample groups were indistinguishable from each other, as all points were grouped under the same area in the DFA canonical plot. Control samples and samples incubated for 6, 8, 10, 12, and 14 h occupied different areas, indicating that the electronic sensors could differentiate between samples with and without *Salmonella* Typhimurium as well as between different numbers of *Salmonella* Typhimurium.

Applying DFA, a good separation between all sample groups was observed. DFA could qualitatively classify the sample but did not provide quantitative information of the number of *Salmonella* Typhimurium present in the sample. Therefore, ANN was used for the prediction of *Salmonella* Typhimurium number.

3.4. ANN for prediction of number of Salmonella Typhimurium

Multilayer perceptron (MLP) neural network based on back propagation was used to predict number of *Salmonella* Typhimurium from the volatile metabolites analyzed using the data from electronic sensor array analysis. The network architecture created for the *Salmonella* Typhimurium data matrix includes an input layer, one hidden layer of neurons, and an output layer (Fig. 4). The indices j, k, and l refer to the input signals (j = 1, . . ., m) in the input layer, the neurons (k = 1, . . ., p) in the hidden layer, and the neuron (l = 1, . . ., q) in the output layer, respectively. There were 12 neurons (m = 12) in the input layer, 10 neurons (p = 10) in the hidden layer, and one neuron (q = 1) in the output layer. The transfer function (φ) in the hidden layer was a hyperbolic tangential sigmoid (Eq. (4)), and a linear function (Eq. (5)), was used in the output layer. The nonlinear

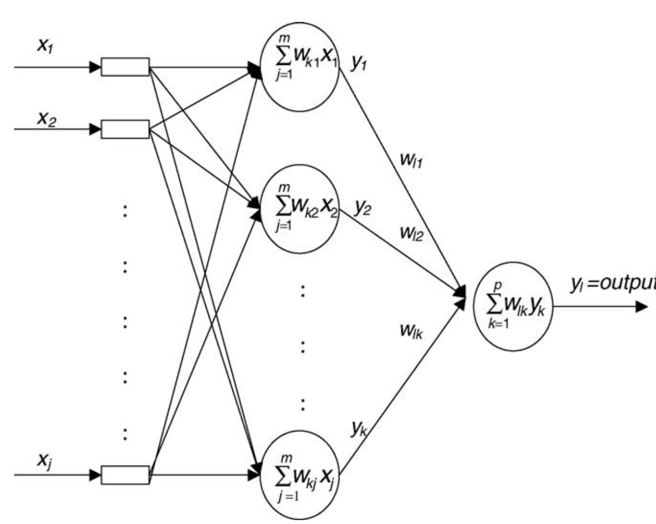

Fig. 4. Multilayer perceptron neural network comprising input layer, one hidden layer, and output layer.

hyperbolic tangent function can be calculated as follows:

$$y_k = \varphi(v_k) = \tanh(b_k + \sum_{j=1}^{m} w_{kj} x_j) \quad (5)$$

where y_k is the output of the hidden layer, $\varphi(v_k)$ the transfer function associated with the neuron k in the hidden layer, v_k the sum of weighted input of neuron k, b_k the bias, and x_j is the input signal [12–14]. A linear function can be calculated as follows:

$$y_l = \varphi(v_l) = b_l + \sum_{k=1}^{p} w_{lk} y_k \quad (6)$$

where y_l is the output of the output layer, $\varphi(v_l)$ the transfer function associated with neuron l in the output layer, y_k the input to the neuron l, v_l the sum of weighted input of neuron l, b_l the bias, and w_{lk} is the weight connection of neuron k and neuron l. The ANN created was trained using selected parameters in data sets from several cultivations and subsequently validated on independent data sets for estimating the concentration variables. The performance function used during training of the neural network was the mean sum of squares of the network errors (MSE).

$$\text{MSE} = \frac{1}{n} \sum_{i=1}^{n} (t_i - a_i)^2 \quad (7)$$

where a is the network output, t the targets and n is the number of samples. The difference between the target value and actual neural output was propagated back through the network to the input. In learning process, the error is minimized by adjusting the weight. The updated weight value is computed by

$$w_{kj}(n+1) = w_{kj}(n) + \eta e_k(n) x_j(n) \quad (8)$$

where $\Delta w_{kj}(n)$ is the weight adjustment applied to the synaptic weight at time step n, e_k the mean square error, x_j the element of the input vector, and η is the learning rate parameter. The learning rate parameter ($\eta = 0.05$) was selected to ensure that the convergence of the learning process was achieved [15–17]. The learning process performed until the error covering the entire training set converged to the minimum value (MSE = 0.021). A low MSE between the predicted and measured values indicates that there was low error when using ANN to predict the number of *Salmonella* Typhimurium. Fig. 5 shows the predictions versus true values of numbers of *Salmonella* Typhimurium in super broth using an ANN. The correlation coefficient, R^2, between the network outputs and the corresponding targets was a measure of how well the variation in the output was explained by the targets and outputs. R^2 was close to 1 ($R^2 = 0.998$), which indicated a good fit.

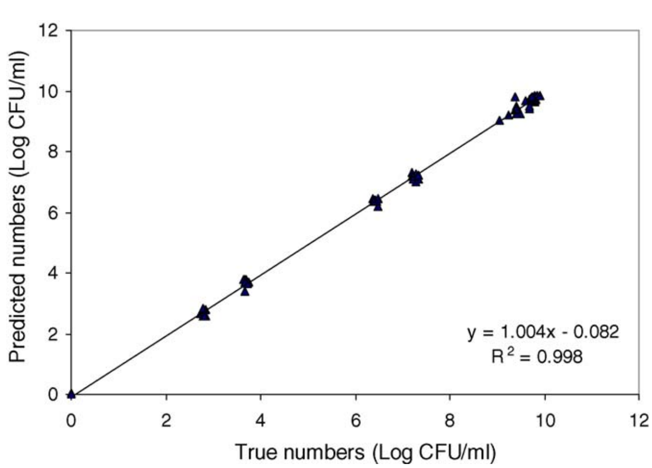

Fig. 5. The predicted and true numbers of *Salmonella* Typhimurium using ANN algorithm.

4. Conclusion

Electronic sensor array technology may have the potential to monitor changes in the volatile composition of samples containing microorganisms. The number of *Salmonella* Typhimurium in samples proved to have an effect on the electronic sensor responses. The results showed a low MSE and a high R^2, between predicted and true values. Electronic sensor array incorporating a MLP neural network has the potential to predict the number of *Salmonella* Typhimurium in samples. Conceptually, the electronic sensor array using rapid sequential analysis has the potential to be used for the analysis of volatiles, whereas ANN becomes a simple and cost-effective tool for predicting the number of *Salmonella* Typhimurium bacteria in food products.

References

[1] J.M. Jay, Modern Food Microbiology, 6th ed., Chapman & Hall, New York, 2000, p. 449.
[2] J.P. Stetter, S. Strathmann, C. McEntegart, M. Decastro, W.R. Penrose, New sensor arrays and sampling systems for a modular electronic nose, Sens. Actuators B: Chem. 69 (2000) 410–419.
[3] L. Marilley, S. Amupuero, T. Zesiger, M.G. Casey, Screening of aroma-producing lactic acid bacteria with electronic nose, Int. Dairy J. 14 (2004) 846–849.
[4] M.P. Mart, O. Busto, J. Guasch, R. Boqué, Electronic noses in the quality control of alcoholic beverages, Trends Anal. Chem. 24 (2005) 57–66.
[5] M.A. Martin, J.P. Santos, J.A. Agapito, Application of artificial neural networks to calculate the partial gas concentration in a mixture, Sens. Actuators B: Chem. 77 (2001) 468–471.
[6] J.A. Ragazzo-Sanchez, P. Chalier, C. Ghommidh, Coupling gas chromatography and electronic nose for dehydration and desalcoholization of alcoholized beverages application to off-flavour detection in wine, Sens. Actuators B: Chem. 104 (2005) 301–308.
[7] K. Brudzewski, S. Osowski, T. Markiewicz, Classification of milk by means of an electronic nose and SVM neural network, Sens. Actuators B: Chem. 98 (2004) 291–298.
[8] D. Lee, J. Jung, J. Lim, J. Hus, D. Lee, Recognition of volatile organic compounds using SnO_2 sensor array and pattern recognition analysis, Sens. Actuators B: Chem. 77 (2001) 228–236.
[9] L. Tomasko, R.W. Helms, S.M. Snapinn, A discriminant analysis extension to mixed models, Stat. Med. 18 (1999) 1249–1260.
[10] Q. Lucas, J. Poling, V. Bennincasa, Analysis with hybrid system PCM/CP/MOS, Semin. Food Anal. 3 (1998) 53–58.
[11] W.K. Fung, Diagnostics in linear discriminant analysis, J. Am. Stat. Assoc. 90 (1995) 952–956.
[12] F.R. Burden, R.G. Brereton, P.T. Walsh, Cross-validation selection of test and validation sets in multivariate calibration and neural network as applied to spectroscopy, Analyst 122 (1997) 1015–1022.

[13] P. Coulibaly, B. Bobée, F. Anctil, Improving extreme hydrologic events forecasting using a new criterion for artificial neural network selection, Hydrol. Process. 15 (2001) 1533–1536.
[14] S. Haykin, Neural Networks: A Comprehensive Foundation, 2nd ed., Prentice Hall, New Jersey, 1999, p. 541.
[15] S. Bila, Y. Harkouss, M. Ibrahim, J. Rousset, E. N'Goya, D. Baillargeat, S. Verdeyme, M. Aubourg, P. Guillon, An accurate wavelet neural-network-based model for electromagnetic optimization of microwave circuits, Int. J. RF Microw. Comput. Aid. Eng. 93 (1999) 297–306.
[16] V. Devabhaktuni, M.C.E. Yagoub, Y. Fang, J. Xu, Q. Zhang, Neural networks for microwave modeling: model development issues and nonlinear modeling techniques, Int. J. RF Microw. Comput. Aid. Eng. 11 (2001) 4–21.
[17] M.H. Terra, R. Tinós, Fault detection and isolation in robotic manipulators via neural networks: a comparison among three architectures for residual analysis, J. Rob. Syst. 18 (2001) 357–374.

Biographies

Ubonrat Siripatrawan obtained her PhD degree in packaging from Michigan State University in 2002. She is a Lecturer in Department of Food Technology, Faculty of Science, Chulalongkorn University. Her research interests cover Electronic sensors, GC–MS and ANN for rapid detection of microorganisms in packaged food products; Food shelf life simulations and Active packaging.

John E. Linz obtained his PhD degree in microbiology from Louisiana State University in 1983. He is a Professor in Department of Food Science and Human Nutrition, Michigan State University. His main research interests are prevention of mycotoxins in the food supply-regulation of mycotoxin biosynthesis; regulation of cell differentiation in filamentous fungi and mechanisms of pathogenesis and antibiotic resistance in food borne pathogens.

Bruce R. Harte obtained his PhD degree in food science and human nutrition from Michigan State University in 1979. He is a Professor in the School of Packaging and an Adjunct Professor in Department of Food Science and Human Nutrition, Michigan State University. He served as a Director, School of Packaging, Michigan State University from 1993 to 2004. His main research interests are Food product/package compatibility and interaction; MAP, CAP of food products; Extension of shelf life and maintenance of food quality through packaging; Microwave heating of product/package systems; Material and container properties and Package development.