Microbial Pathogenesis 67-68 (2014) 36–40

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Genetic algorithm-artificial neural network and adaptive neuro-fuzzy inference system modeling of antibacterial activity of annatto dye on *Salmonella enteritidis*

Mahmoud Yolmeh [a,*], Mohammad B. Habibi Najafi [b], Fakhreddin Salehi [a]

[a] Faculty of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[b] Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

A R T I C L E I N F O

Article history:
Received 16 January 2014
Received in revised form
8 February 2014
Accepted 10 February 2014
Available online 22 February 2014

Keywords:
Annatto dye
Genetic algorithm
Mayonnaise
Salmonella enteritidis
Sensitivity analysis

A B S T R A C T

Annatto is commonly used as a coloring agent in the food industry and has antimicrobial and antioxidant properties. In this study, genetic algorithm-artificial neural network (GA-ANN) and adaptive neuro-fuzzy inference system (ANFIS) models were used to predict the effect of annatto dye on *Salmonella enteritidis* in mayonnaise. The GA-ANN and ANFIS were fed with 3 inputs of annatto dye concentration (0, 0.1, 0.2 and 0.4%), storage temperature (4 and 25 °C) and storage time (1–20 days) for prediction of *S. enteritidis* population. Both models were trained with experimental data. The results showed that the annatto dye was able to reduce of *S. enteritidis* and its effect was stronger at 25 °C than 4 °C. The developed GA-ANN, which included 8 hidden neurons, could predict *S. enteritidis* population with correlation coefficient of 0.999. The overall agreement between ANFIS predictions and experimental data was also very good ($r = 0.998$). Sensitivity analysis results showed that storage temperature was the most sensitive factor for prediction of *S. enteritidis* population.

1. Introduction

Salmonellosis is the first most reported infectious disease in Brazil, the third in the US, and often associated with human illness in the European Union [19]. *S. enteritidis* is an enteropathogen, particularly in foods that do not require heat treatment before consumption such as salads prepared with mayonnaise [2]. Mayonnaise is one of the oldest sauces that is widely consumed around the world. Preservatives are being added to mayonnaise to compensate the insufficient heat treatment in the processing. Mayonnaise is the most common cause of salmonellosis outbreaks worldwide [23]. Using of natural alternatives instead of synthetic preservatives is attractive due to the fact that synthetic preservatives are believed to be non-safe and potentially harmful in the long-term.

Annatto dye contains a series of carotenoid-type pigments extracted from seeds of a bush (*Bixa orellana* L.), which imparts a yellow to red color to food [12]. Antimicrobial activity of annatto extract is due to the presence of several mono and sesquiterpenes [15].

Da Silva and Franco [5]; studied the application of oregano essential oil against *S. enteritidis* in mayonnaise salad, they observed that the number of *S. enteritidis* was decreased by adding oregano essential oil into mayonnaise.

While the kinetics of microbial heat inactivation have been studied extensively, there has been relatively little information reported on quantitative data of non-thermal inactivation. In the former case, the rate of bacterial inactivation has usually been determined using linear regression (log number survivors versus time). However, recently it has been indicated that many bacterial inactivation curves are not linear [11].

The majority of the microbial inactivation models are lying in the second and third category due to the lack of sufficient microbial/biochemical knowledge concerning the inactivation of microorganisms [20].

The non-linear techniques of Artificial Neural Networks (ANN) and adaptive neuro-fuzzy inference system (ANFIS) lie in the black box modeling group. Researchers explored the potential of ANN and ANFIS as an analytical alternative to conventional modeling techniques, which are often limited by strict assumptions of normality, linearity, homogeneity, and variable independence [20]; [16]. ANNs are information processing networks constituting a set of highly interconnected neurons arranged in multiple layers that

* Corresponding author. Tel.: +98 9368171909, fax: +98 1735228573.
E-mail addresses: mahmud.yolmeh@yahoo.com (M. Yolmeh), habibi@ac.um.ir (M.B. Habibi Najafi), Fs1446@yahoo.com (F. Salehi).

http://dx.doi.org/10.1016/j.micpath.2014.02.003
0882-4010/

can be trained to fit one or more dependent variables to any degree of accuracy using a set of independent variables as inputs [9]. Fuzzy inference systems (FIS) and ANNs are model-free numerical estimators. In order to utilize the strengths of both, FISs and ANNs may be combined into an integrated system called ANFIS; the integrated system then has the advantages of both ANNs (e.g., learning abilities, optimization abilities, and connectionist structure) and FISs (e.g., humanlike if-then rules, and ease of incorporating expert knowledge available in linguistic terms) [16].

Neuro-fuzzy systems are of great importance among various combinations of methodologies in soft computing [6]. Neuro-fuzzy uses neural network learning functions to refine each part of the fuzzy knowledge separately. Learning in a separated network is faster than learning in a whole network. One approach to the derivation of a fuzzy rule base is to use the self-learning features of artificial neural networks, to define the membership function (MF) based on input–output data [7]. The determination of MF parameters and fuzzy rules is not easy for more complex problems. ANFIS structure gives an easy way to generate the MFs and fuzzy rules for Sugeno-type fuzzy inference systems [8].

These techniques have been used for accurately describing the interacting effect of extrinsic/intrinsic factors on the microbial growth kinetics. Lou and Nakai [14] proposed an ANN for studying the effect of temperature, pH and a_w on the thermal inactivation rate of *E. coli*. The methodology generated accurate results when compared with other secondary models. Additionally, the use of ANNs as an integrated primary-secondary inactivation model can contribute in an overall approach for modeling the microbial inactivation dynamics [4].

However, there is no study available in the literature concerning the use of computing technology for prediction of annatto dye on *S. enteritidis*. Hence, the objectives of this work were to investigate the effect of annatto dye concentration, storage temperature and storage time on *S. enteritidis* and study the efficiency of GA-ANN and ANFIS for microbial inactivation modeling.

2. Materials and methods

2.1. Materials

Annatto seeds were purchased from the market of Hyderabad, India. Organic solvents and media used were all analytical grades and obtained from Merck, Germany. *S. enteritidis* was kindly donated by Ferdowsi University of Mashhad, Dept. of Veterinary.

2.2. Dye extraction

Dye extraction was done according to the method of Castello et al. [3]. Briefly, annatto seeds were soaked in *n*-hexane for 6 h in order to remove oils, dye was then extracted by acetone from defatted seeds. After filtration through Whatman filter paper (No. 1), the extract was concentrated by rotary evaporation and then vacuum and then vacuum-dried in the 1410D-2E vacuum oven (Shel Lab, USA) to produce dye powder.

2.3. Mayonnaise preparation

Preservative free mayonnaise was produced in the laboratory. Yolk and 4% acetic acid was used as emulsifier and as an acidic agent, respectively. pH of the mayonnaise was 4.4. Annatto extract was added to the mayonnaise formulation to final concentrations of 0.1, 0.2 and 0.4 % (v/v). Bacterial suspension was inoculated into each sample to obtain a final concentration of 1.5×10^8 cfu/g. Samples were stored at room (25 °C) or refrigerator (4 °C) temperature.

2.4. Determination of antimicrobial activity

For this purpose, 10 g of mayonnaise was dissolved in 90 ml of ringer and a series of 10-fold dilutions was prepared from this suspension and subsequently used for plating on plate count agar [5].

2.5. GA-ANN model

ANN is a type of artificial intelligence that mimics the behavior of the human brain and it has attained its popularity due to the use of a generalization technique instead of memorization [6]. The most popular ANN is the multi-layer feed-forward neural network, where the neurons are arranged into three layers of input, hidden and output. A schematic description of the 3-layers network structure used in this study is shown in Fig. 1. The performance of an ANN depends strongly upon its topology. The number of input neurons corresponds to the number of input variables into the neural network, and the number of output neurons is similar to the number of target output variables. Between the input and the output layers, there is at least one hidden layer, which can have any number of neurons and depends on the application of the network. Determination of optimum number of hidden layer neurons is usually performed by trial and error method [1,21].

Genetic algorithm (GA) optimization technique can be used to overcome this inherent limitation of ANN. GA are search techniques for an optimal value, mimicking the mechanism of biological evolution. They have a high ability to find an optimal value (global optimal value or at least near global one) of a complex objective function, without falling into local optima [17,22].

In the hidden and output layers, the net input (x_j) to node j is of the form:

$$X_j = \sum_{i=1}^{n} W_{ij} y_i + b_j \quad (1)$$

where y_i are the inputs, w_{ij} are the weights associated with each input/node connection, n is the number of nodes and b_j is the bias associated with node j. Additionally, bias is an extra input added to neurons. The reason for adding the bias term is that it allows a representation of phenomena having thresholds [22]. A hyperbolic tangent activation function (Eq. (2)) was chosen to use as the transfer function in the hidden and output layers.

$$f(x) = \frac{e^x - e^{-x}}{e^x + e^{-x}} \quad (2)$$

In the present study, 250 data were collected from experiments and then all data were randomly divided into 3 partitions: training (30%), validating (10%), and testing data (60%). The testing data were used for estimating the performance of the trained network

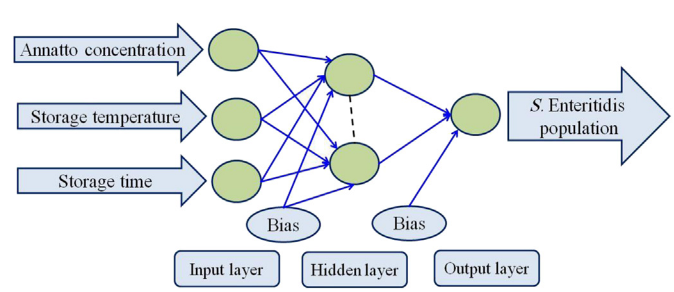

Fig. 1. ANN architecture with one hidden layer for prediction of *S. enteritidis* population.

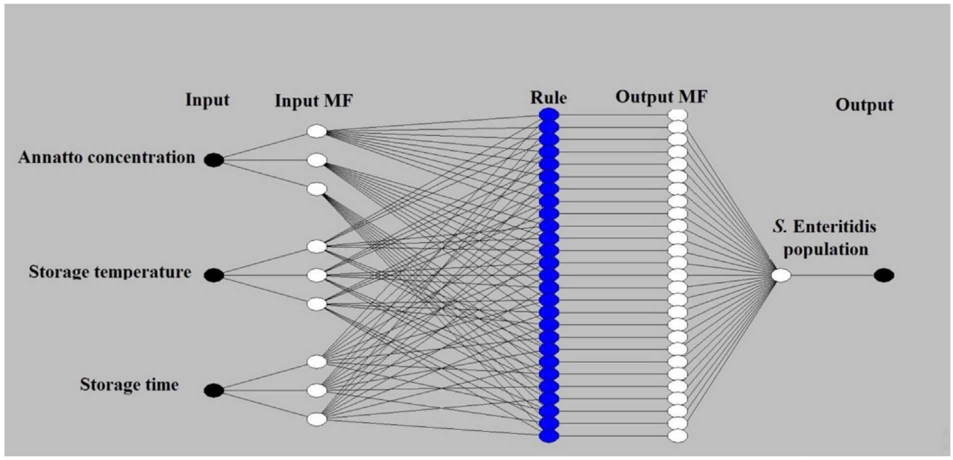

Fig. 2. The general structure of ANFIS for the *S.* Enteritidis population model with 3 inputs.

on new data, which never was seen by the network during the training (unseen data). The probabilities of the crossover and mutation operators were adjusted at 0.9 and 0.01, respectively.

In addition, a sensitivity analysis was conducted to provide a measure of the relative importance among the inputs of the neural network model and to illustrate how the model output varied in response to variation of an input [1]. In this work, the Neurosolution software (release 6.01, NeuroDimension, Inc., USA) was used for designing the GA-ANN model.

2.6. ANFIS model

For premise parameters that define MFs, ANFIS employs gradient descent back-propagation neural networks to fine-tune them. A hybrid training method (the combination of least-squares and back-propagation algorithms) was used as the training method of the ANFIS.

ANFIS modeling was started by obtaining a data set (input–output data points). The data order was the first randomized and then all data were separated into three partitions: 30, 10 and 60% of total data were used for training, validating and testing (unseen data) the network, respectively. Each input/output pair contained three inputs (dye concentration, storage temperature and storage time) and one output (*S. enteritidis* population) (Fig. 2.). The number of MFs assigned to each input variable is chosen by trial and error. The ANFIS toolbox of Matlab 7.6 was used to obtain the results, and to build an ANFIS model for predicting the *S. enteritidis* population.

3. Results and discussion

3.1. Effect of annatto on S. enteritidis

The results showed that the total count of *S. enteritidis* in samples containing annatto extract at the level of 0.1 and 0.2% was reduced from an initial value of 1.5×10^8 cfu/g to 5.11 and 4.62 cfu/g, respectively, as it is evident in Fig 3. Since the reduction of *S. enteritidis* in the control samples due to the effect of acidic conditions alone was much less than dye treated samples, it can be concluded that annatto extract with a strong influence on *S. enteritidis* could be successfully used in the production of safe and preservative free mayonnaise.

Fig. 4 shows the effect of storage temperature on total count of *S. enteritidis* in mayonnaise. *S. enteritidis* was more rapidly decreased in samples stored at 25 °C than at 4 °C. No *S. enteritidis* was found after 17 days of inoculation at 25 °C whereas *S. enteritidis* population reached about 5.5 cfu/g after 20 days of inoculation in samples stored at 4 °C. The same finding was observed by Perales and Garcia [18].

Heo et al. [10] studied the inactivation of *Salmonella typhimurium* during storage of the commercial mayonnaise at different temperatures and pH. More declines in the viability of *S. typhimurium* was

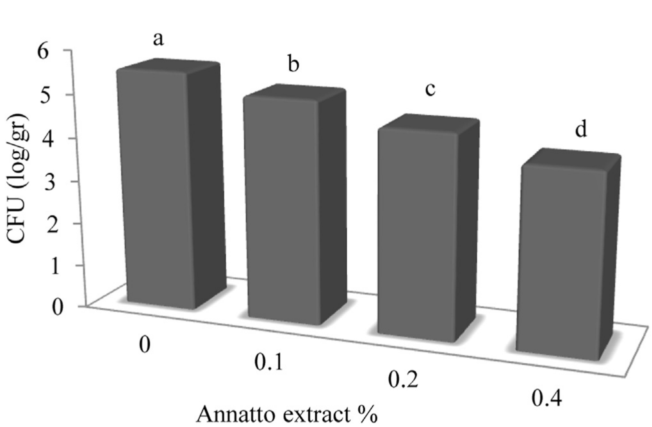

Fig 3. Effect of annatto extracts on *S. enteritidis* growth in mayonnaise.

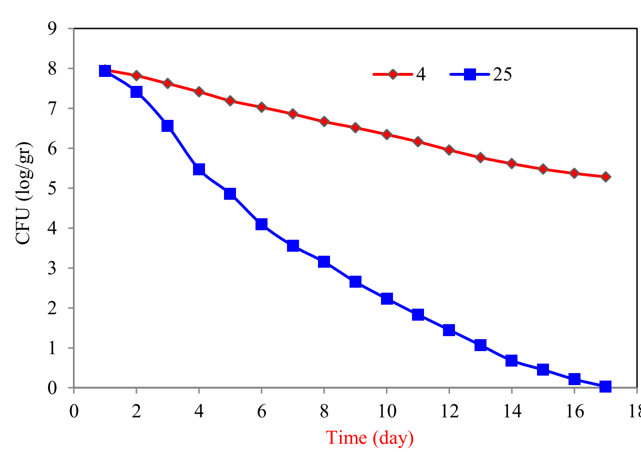

Fig 4. Effect of the temperature on *S. enteritidis* in mayonnaise with added annatto extract during storage.

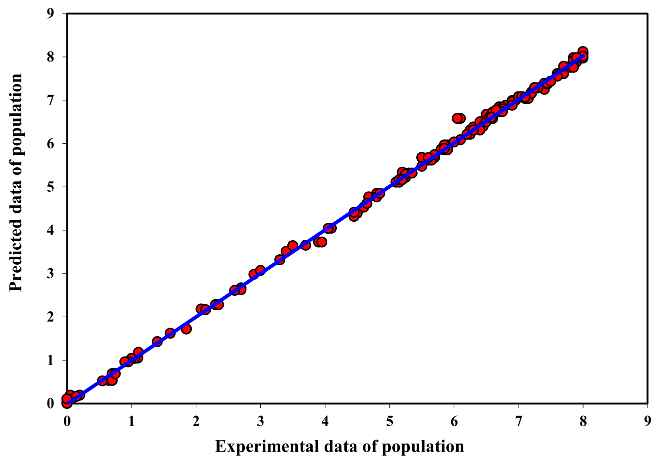

Fig. 5. Experimental versus predicted values of *S. enteritidis* population using GA-ANN model for the test data set ($r = 0.999$).

observed in samples stored at 40 °C than 10 °C. Similar result was found by Lock and Board [13] during the study of viability of *S. enteritidis* in different mayonnaises.

3.2. GA-ANN

GA-ANN model was developed for estimation of *S. enteritidis* population. In this study, ANN with 2–20 neurons was trained using GA to find the optimal network configuration. It was found that GA-ANN with 8 neurons in one hidden layer could predict *S. enteritidis* population with high correlation coefficient ($r = 0.999$). The prediction efficiency of the GA-ANN model for unseen data is presented in Fig. 5. The calculated correlation coefficient value for estimation of *S. enteritidis* population shows high correlation between predicted and experimental values. Table 1 illustrates the weights and bias values of the optimized network, which could be applied in a computer program for estimation of *S. enteritidis* population in mayonnaise. The results showed that an acceptable agreement between the predicted and experimental data could be achieved using GA-ANN model.

Sensitivity analysis was also used in order to study the sensitiveness of neural network models toward different inputs (Fig. 6). Among the input variables, storage temperature was the most sensitive factor, followed by storage time and finally annatto concentration for prediction of *S. enteritidis* population by the selected GA-ANN.

3.3. ANFIS

The ANFIS network parameters, such as the type and number of MF and epochs, have been varied to obtain the best results in terms

Table 1
The weights and bias values of optimized GA-ANN model.

Hidden neurons	Bias	Input neurons			Output neurons
		Annatto concentration	Storage temperature	Storage time	*S. enteritidis* population
1	2.152	−0.235	−2.365	0.356	0.895
2	1.023	1.235	1.325	0.365	0.685
3	−1.325	1.325	1.356	−1.235	−1.658
4	0.365	0.365	1.36514.	1.352	0.658
5	0.365	1.236	1.365	0.325	0.325
6	0.652	0.325	0.365	0.985	1.365
7	1.235	2.365	0.985	0.986	−1.356
8	−3.253	0.235	0.685	1.759	−2.356
Bias					−1.256

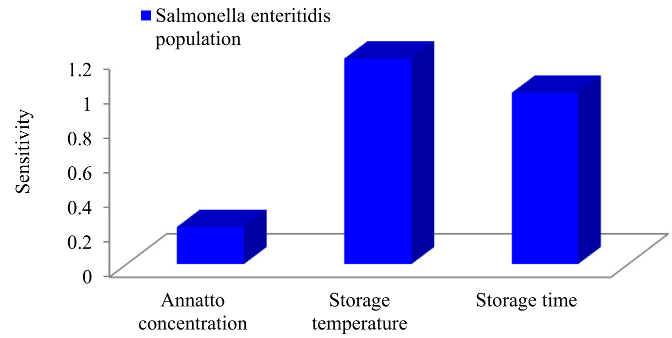

Fig. 6. Sensitivity analysis of optimized GA-ANN (3/8/1) for prediction of *S. enteritidis* population.

of model validation. ANFIS architecture used in this study is shown in Fig. 2. The final ANFIS architecture for predicting the *S. enteritidis* population, with three Gaussians type MFs for each input (3 inputs) and linear MF for output, and constructed 27 rules resulted high accurate prediction. In Fig. 7 the *S. enteritidis* values versus ANFIS predictions for test data (unseen data) points are shown. It can be seen that the system was well-trained to model the population of *S. enteritidis* ($r = 0.998$).

4. Conclusion

Mayonnaise that contains 0.4% of annatto extract could decrease the population of *S. enteritidis*. The survival curve of *S. enteritidis* in mayonnaise reveals that the population in samples stored at 4 °C up to 20 days after inoculation were not reduced to zero whereas the population in samples stored at 25 °C reached zero within 17 days after inoculation. According to the results, annatto dye with its antimicrobial activity can be recommended to be used as an alternative to synthetic preservatives in the formulation of mayonnaise. From a food quality and safety point of view, prevention is a better strategy than detoxification, which is much more complicated.

The application of GA-ANN and ANFIS to simulation of *S. enteritidis* population in mayonnaise was investigated to predict the population (output) versus annatto concentration, storage temperature and time (inputs). It was found that GA-ANN with 1 hidden layer comprising 8 neurons gives the best fitting with the experimental data, which made it possible to predict *S. enteritidis* population with an acceptable correlation coefficient (0.999). It was also found that ANFIS models with three Gaussian type MFs

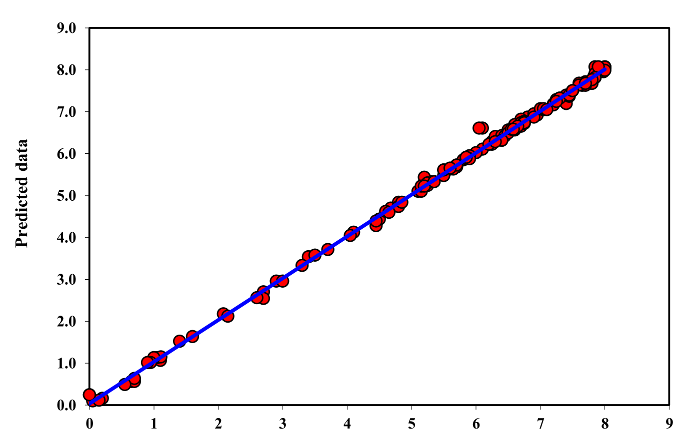

Fig. 7. Experimental versus predicted values of *S. enteritidis* population using ANFIS model for the test data set ($r = 0.998$).

(gussmf) for all input variables and linear for output gives the best fitting with the experimental data, which made it possible to predict *S. enteritidis* population with a high correlation coefficient (0.999). The results indicated that both GA-ANN and ANFIS models could give good prediction for population of *S. enteritidis*.

References

[1] BahramParvar M, Salehi F, Razavi SMA. Predicting total acceptance of ice cream using artificial neural network. J Food Process Preserv; 2013:1–9.

[2] Boonmar S, Bangtrakulnonth A, Pornrunangwong S, Terajima J, Watanabe H, Kaneko K, et al. Epidemiological analysis of *Salmonella enteritidis* isolates from humans and broiler chickens in Thailand by phages typing and pulsed-field gel electrophoresis. J Clin Microbiol 1998;36(4):971–4.

[3] Castello M, Chandra N, Phatak A, Madhuri S. Estimation of bixin in seeds of Bixa orellana L. from different locations in Western Maharashtra. Indian J Plant Physiol 2004;9(2):185–8.

[4] Cheroutre-Vialette M, Lebert A. Application of recurrent neural network to predict bacterial growth in dynamic conditions. Int J Food Microbiol 2002;73:107–18.

[5] Da Silva JPL, Franco BDG. Application of oregano essential oil against *salmonella enteritidis* in mayonnaise salad. Int J Food Sci Nutr Eng 2012;2(5):70–5.

[6] Entchev E, Yang L. Application of adaptive neuro-fuzzy inference system techniques and artificial neural networks to predict solid oxide fuel cell performance in residential microgeneration installation. J Power Sources 2007;170:122–9.

[7] Ghoush MA, Al-Mahasneh M, Samhouri M, Al-Holy M, Herald T. Formulation and fuzzy modeling of viscosity of orange-flavored beverages fortified with carboxymethylcellulose-whey protein isolate emulsions. Jordan J Biol Sci 2009;2(3):109–18.

[8] Gulbag A, Temurtas F. A study on quantitative classification of binary gas mixture using neural networks and adaptive neuro-fuzzy inference systems. Sens Actuators B 2006;115:252–62.

[9] Haykin S. Neural networks: a comprehensive foundation. Englewood Cliffs, NJ: Macmillan Publishing Company; 1994. p. 696.

[10] Heo SK, Lee JY, Jo SH, Bae DH, Kim YS, Chung MS, et al. Inactivation of Salmonella enterica serovar *typhimurium* in commercial mayonnaise at different temperatures and pH. J Korean Soc Appl Biol Chem 2010;53(3):351–5.

[11] Koutsoumanis K, Lambropoulou K, Nychas G-JE. A predictive model for the non-thermal inactivation of *Salmonella enteritidis* in a food model system supplemented with a natural antimicrobial. Int J Food Microbiol 1999;49:63–74.

[12] Lauro GJ. A primer on natural colors. Cereal Foods World 1991;36:949–53.

[13] Lock JL, Board RG. The fate of *Salmonella enteritidis* PT4 in deliberately infected commercial mayonnaise. Food Microbiol 1994;11:499–504.

[14] Lou W, Nakai S. Application of artificial neural networks for predicting the thermal inactivation of bacteria: a combined effect of temperature, pH and water activity. Food Res Int 2000;34:573–9.

[15] Magiatis P, Melliou E, Skaltsounis A, Chinou I, Mitaku S. Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. chia. Planta Med 1999;65:749–52.

[16] Mashrei MA, Abdulrazzaq N, Abdalla TY, Rahman MS. Neural networks model and adaptive neuro-fuzzy inference system for predicting the moment capacity of ferrocement members. Eng Struct 2010;32:1723–34.

[17] Morimoto T. Genetic algorithm. In: Sablani SS, Rahman MS, Datta AK, Mujumdar AS, editors. Handbook of food and bioprocess modeling techniques. New York: CRC Press; 2006. pp. 405–34.

[18] Perales I, Garcia MI. The influence of pH and temperature on the behaviour of *Salmonella enteritidis* phage type 4 in home-made mayonnaise. Lett Appl Microbiol 1990;10:19–22.

[19] Rajashekara G, Munir S, Alexeyev MF, Halvorson DA, Wells CL, Nagaraja KV. Pathogenic role of SEF14, SEF17 and SEF21 fimbriae in *Salmonella enterica* serovar *enteritidis* infection of chickens. Appl Environ Microbiol 2000;66(4):1759–63.

[20] Rumelhart DE, Durbin R, Golden R, Chauvin Y. Backpropagation: theory, architectures, and applications backpropagation: the basic theory. New Jersey: Lawrence Erlbaum Associates; 1994. pp. 1–34.

[21] Salehi F, Razavi SMA. Dynamic modeling of flux and total hydraulic resistance in nanofiltration treatment of regeneration waste brine using artificial neural network. Desalin Water Treat 2012;41:95–104.

[22] Salehi F, Razavi SMA. Applying the genetic algorithm–artificial neural network modeling for prediction of permeate flux, NaCl rejection and total hydraulic resistance of nanofiltration process. J Sep Transport Phenom 2011;22(1):29–50.

[23] Smittle RB. Microbiological saftey of mayonnaise, salad dressing and sauces produced in the United States. J Food Prot 2000;63(8):1144–53.