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ORIGINAL ARTICLE

New approaches to modeling *Staphylococcus aureus* inactivation by ultrasound

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Abstract Ultrasound (US) is an effective technology to inactivate vegetative microorganisms in foods. In this study, the effect of amplitude levels (0.4, 7.5, and 37.5), duty cycles (0.3:0.7 s, 0.7:0.3 s, and 0.9: 0.1 s) and time (0, 2, 4, 6, 8, 10, 12, and 14 days) of US on inactivation of *Staphylococcus aureus* were investigated. In addition, genetic algorithm-artificial neural network (GA-ANN) and adaptive neuro-fuzzy inference system (ANFIS) models were used to predict inactivation of *S. aureus*. The GA-ANN and ANFIS were fed with three inputs of amplitude levels, duty cycles, and time. The inactivation rate of *S. aureus* was increased by increasing the amplitude levels, and the best inactivation was obtained at a 37.5 μm amplitude for which the *S. aureus* population was reduced to 2.59 CFU/mL. The high inactivation of *S. aureus* was achieved under a duty cycle of 0.7:0.3 s with reduction of the population to 1.49 CFU/mL. The developed GA-ANN, which included 17 hidden neurons, could predict the *S. aureus* population with a coefficient of determination of 0.986. The overall agreement between ANFIS predictions and experimental data was also very good (R^2=0.979). Sensitivity analysis results showed that the amplitude level was the most sensitive factor for prediction of *S. aureus*.

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Introduction

The food industry is seeking to develop alternative processing technologies to produce foods without detrimental changes of physicochemical, nutritional, and organoleptic properties induced by the technologies themselves whilst preserving microbial safety profiles (Esteve and Frigola 2007).

Ultrasound (US) is currently used in a range of industries, for example, in food and beverage processing, surface cleaning, medical scanning, nanotechnology, mineral processing, welding, and nondestructive testing (Joyce et al. 2007; Mason 2007). Assisted US processes are expected to enhance the safety of fresh and processed foods and extend their shelf life, while preserving or improving organoleptic properties (Sango et al. 2014). The efficiency of US as a disinfection method can be increased by combining it with another technique such as heat, pressure, UV radiation, and pulsed electric fields (PEF) (Chemat and Khan 2011; Awad et al. 2012). In addition, assisted US is more energy efficient and because lower intensities for shorter times can be used, it can have a positive influence on some food characteristics such as the appearance in milk; as well as to enhance efficiency at the industrial level (Demirdoven and Baysal 2008; Bermudez-Aguirre et al. 2009).

Mechanical vibrations with frequencies higher than 15 kHz create US waves. Alternating compression and expansion cycles are generated by these waves in liquid media. US waves create small bubbles in liquid during irradiation. When these bubbles attain a volume at which they can no higher absorb enough energy, they burst strongly. This phenomenon is known as cavitation (Rodgers and Ryser 2004). Inside these bubbles can be very high temperatures (nearly 5500 °C) and

pressures (nearly 50 MPa) during implosion (Raso et al. 1998). In most authors' precepts, this is the final reason for the bactericidal effect of high intensity US (Ananta et al. 2005; Ugarte-Romero et al. 2007; Joyce et al. 2007).

Microbial inactivation by thermal treatment does damage to the organoleptic properties of foods. However, use of US in order to have microbial inactivation minimizes this change. Generally, the advantages of US over thermal treatment are a smaller flavor loss, significant energy savings, and greater homogeneity (Chemat and Khan 2011; Kiang et al. 2012).

Staphylococcus aureus, a Gram-positive, nonspore-forming, anaerobic facultative rod, is a public concern because of its implication in illness outbreaks (Oonmetta-aree et al. 2006).

Artificial Neural Networks (ANN) and adaptive neuro-fuzzy inference systems (ANFIS) are as an analytical alternative to conventional modeling techniques, which are frequently limited by strict assumptions of normality, linearity, homogeneity, and variable independence. Fuzzy inference systems (FIS) and ANNs are model-free numerical estimators. To use the effectiveness of both, FISs and ANNs could be combined into an integrated system called ANFIS; the integrated system then has the utility of both ANNs and FISs (Rumelhart et al. 1994; Soleimanzadeh et al. 2014; Yolmeh et al. 2014b).

Yolmeh et al. (2014a) used GA-ANN and ANFIS models for prediction of antibacterial activity of annatto dye on *Salmonella enteritidis* in mayonnaise. Their results showed that both GA-ANN and ANFIS models could give good predictions for fate of the *S. enteritidis*.

There is no study available in the literature relating to the use of computing technology for prediction of *Staphylococcus aureus* inactivation by US. Therefore, the first goal of this study was to investigate the effect of US waves on the *S. aureus* population. The second goal was studying the performance of GA-ANN and ANFIS models to simulate this microbial inactivation.

Materials and methods

Bacterial strains and growth conditions

Staphylococcus aureus ATCC 25923 was obtained from the microbiology stock culture, Department of Food Science and Technology, Ferdowsi University of Mashhad, Iran. The strains were stored as frozen stocks at –70 °C in the form of protective beads (Technical Services Consultants Ltd, UK), which were plated onto trypticase soy agar (TSA, Scharlau Chemie, Barcelona, Spain) and incubated overnight at 37 °C to obtain single colonies before storage at 4 °C. Then, a single colony was inoculated into trypticase soy broth (TSB, Scharlau Chemie, Barcelona, Spain) and incubated overnight at 37 °C. Working cultures were obtained from this subculture, adjusted to 0.5 McFarland turbidity, and serially diluted (1/1000) to obtain the required concentration of 10^6 CFU/mL in TSB.

US treatment

Samples (50 mL) were sonicated in a 100 mL glass beaker using a VC750 US generator (Sonics and Materials, Inc., Newtown, Conn., USA) fitted with an autoclavable 13 mm diameter US probe attached to an US transducer. Samples were processed at a constant frequency of 20 kHz. The measurement of the amplitude as an indication of the US cavitation is reported to be a reliable method for indication of the US power (Tsukamoto et al. 2004; Patil et al. 2009). Before and after each experiment, the US probe was sterilized by washing with Virkon (DuPont), followed by thorough rinsing with sterile water. Amplitude levels of 0.4 μm, 7.5 μm, and 37.5 μm with duty cycle (pulse durations) of 0.3:0.7 s, 0.7:0.3 s, and 0.9:0.1 s (on time:off time) and a total time of 1 s were applied for up to 14 min. Off time for the US device was considered to burst the bubbles that were generated by sonication. An ice bath was used to dissipate the heat generated during US treatment, and temperatures were maintained below 30 °C.

Enumeration of surviving bacteria

Samples were removed for analysis at 2 min intervals and serially diluted. The 0.1 mL aliquots of appropriate dilutions were cultured on TSA and incubated at 37 °C for 24 h. Survival curves for each US treatment were plotted. All experiments were repeated in triplicate.

GA-ANN model

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 (Soleimanzadeh et al. 2014). 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 the optimum number of hidden layer neurons is usually performed by a trial and error method (Bahramparvar et al. 2014; Soleimanzadeh et al. 2014). The 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 nearly a global one) of a complex

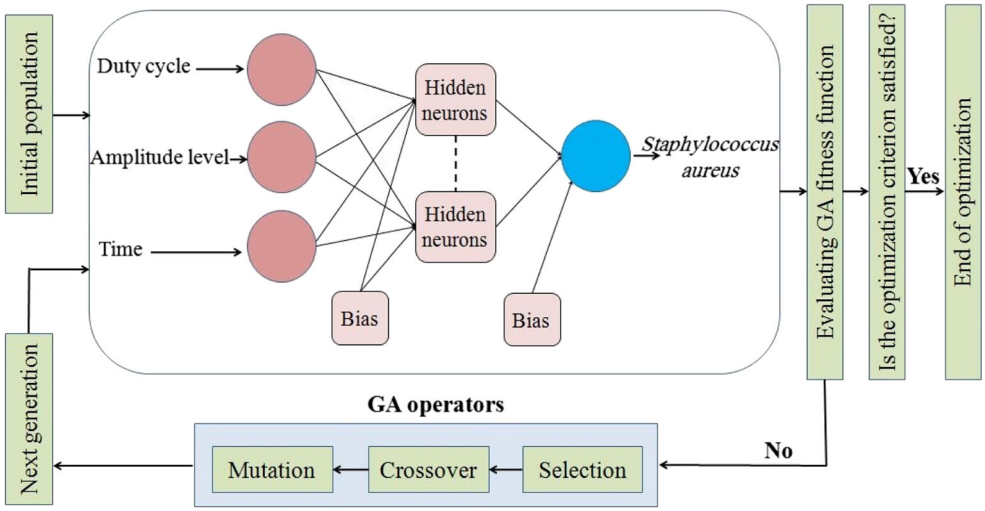

Fig. 1 GA-ANN architecture with one hidden layer for prediction of *S. aureus*

objective function, without falling into local optima (Yolmeh et al. 2014b).

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, the bias is an extra input added to neurons (Soleimanzadeh et al. 2014). A sigmoid activation function (Eq. 2) was chosen to use as the transfer function in the hidden and output layers.

$$f(x) = \frac{1}{1 + e^{-x}} \quad (2)$$

In the present study, 198 data were collected from experiments, and then all data were randomly divided into three partitions: training (20 %), validating (20 %), and testing data (60 %). The testing data was used for estimating the performance of the trained network 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 to be 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 (Soleimanzadeh et al. 2014). In this work, the Neurosolution software (release 6.01, NeuroDimension, Inc., USA) was used for designing the GA-ANN model.

ANFIS model

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 (Yolmeh et al. 2014a). 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 (Yolmeh et al. 2014a). ANFIS modeling was started by obtaining a data set (input–output data points). The data order was first randomized and then all data were separated into three partitions. Of the total data, 20, 20, and 60% was used for training, validating, and testing (unseen data) the network, respectively. Each input/output pair contained three inputs (amplitude level, duty cycle, and time) and one output (*S. aureus* population) (Fig. 2). The number of MFs assigned to each input variable is chosen by trial and error. The ANFIS toolbox of Matlab (version 7.6, Inc., USA) was used to obtain the results, and to build an ANFIS model for predicting the *S. aureus* population.

Results and discussion

Effect of US amplitude level on inactivation of *S. aureus*

The inactivation of *S. aureus* was found to be dependent on the amplitude levels ($p<0.05$). As shown in Fig. 3, the inactivation rate of *S. aureus* was increased by increasing the amplitude levels. The best inactivation was obtained at 37.5 μm amplitude for which the *S. aureus* population was reduced to 2.59 CFU/mL. This is probably due to enhancement of damaging the cell wall and membrane under a greater amplitude value (Piyasena et al. 2003). Hence, sensitivity of the

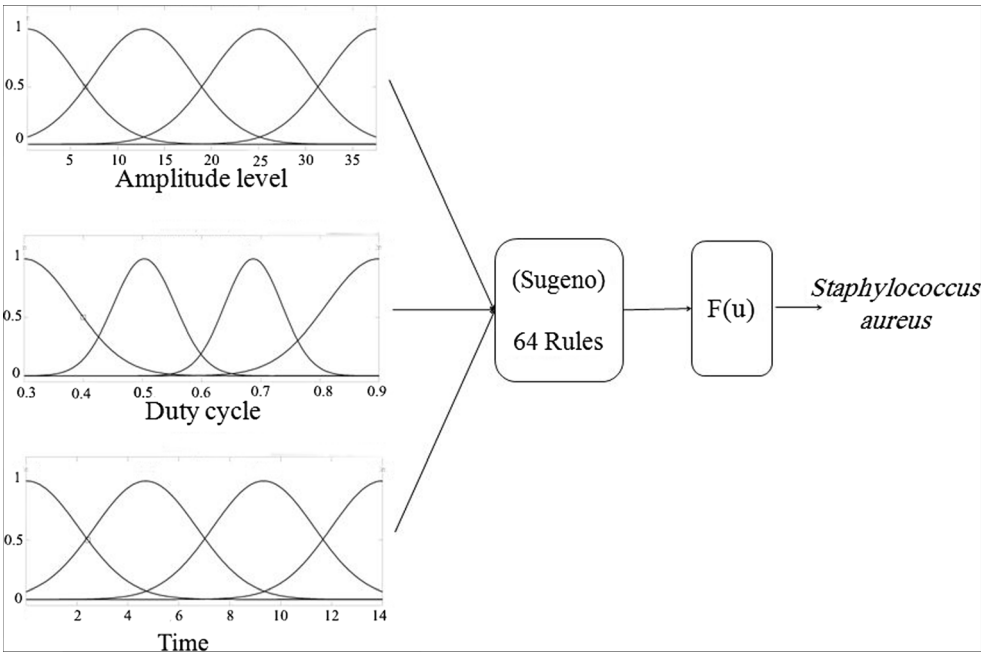

Fig. 2 The general structure of ANFIS for the *S. aureus* population model with three inputs

S. aureus increased in this condition, and this led to increased death of the microorganism. This finding is in agreement with the observation of Patil et al. (2009) and Adekunte et al. (2010) on *Escherichia coli* and *Cronobacter sakazakii*, respectively.

During US treatment, a linear response with exposure time was observed. Similar behavior was observed on the survival curves of *E. coli* (Patil et al. 2009), *Streptococcus mutans* (Koda et al. 2009), *Lactobacillus rhamnosus* (Ananta et al. 2005), and *C. sakazakii* (Adekunte et al. 2010).

As shown in Fig. 4, total inactivation of *S. aureus* cells was achieved using 37.5 μm amplitude after 14 min whereas the *S. aureus* population was reduced to 4.67 and 1.59 CFU/mL under 0.4 and 7.5 μm amplitude after the same time, respectively, as is shown in Fig. 4.

The *S. aureus* destruction curves exhibited a linear relationship in the semi logarithm coordinates under the treatment conditions used in this study, which allows the use of kinetic parameter *D*-values to describe the inactivation behavior. The *D*-values of *S. aureus* inactivation were 10.52 min, 3.17 min, and 2.34 min under 0.4 μm, 7.5 μm, and 37.5 μm amplitude levels, respectively. According to the results, the best inactivation was achieved at a 37.5 μm amplitude with a *D*-value of 2.34 min. Hence, it is possible to increase the amplitude level to achieve a 5-log (t_{5d}) reduction required by the FDA in a relatively short period at sublethal temperatures. The t_{5d} of *S. aureus* were 52.6 min, 15.85 min, and 11.7 at 0.4 μm, 7.5 μm, and 37.5 μm amplitude levels, respectively. T_{5d} for *E. coli* ATCC 25922 was reported to be 11.1 min under 37.5 μm

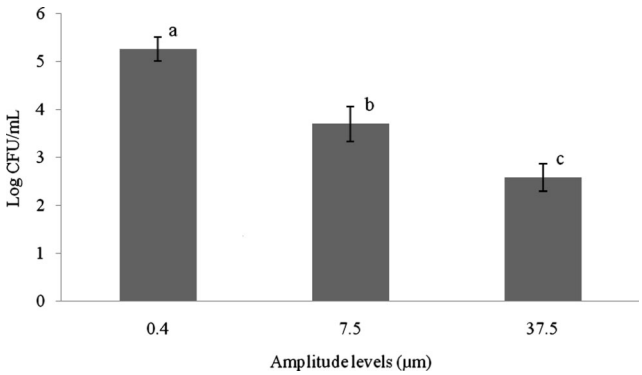

Fig. 3 Effect of amplitude levels on *S. aureus* survival (The initial cell concentration and the length of treatment were 10^6 and 14 min, respectively)

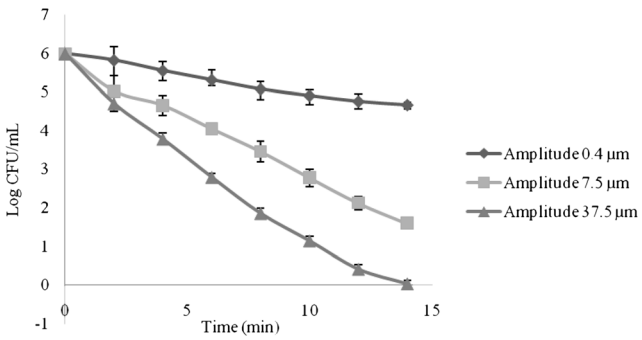

Fig. 4 Effect of amplitude levels on the inactivation of *S. aureus* during US irradiation

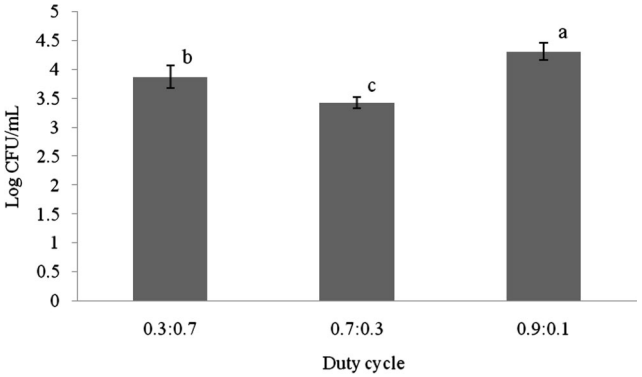

Fig. 5 Effect of duty cycle (on:off time) levels on *S. aureus* survival (The initial cell concentration and the length of treatment were 10^6 and 14 min, respectively)

amplitude by Patil et al. (2009); that is less than the t_{5d} of *S. aureus*. Because of this fact, there is probably a greater thickness of the cell wall of gram-positive bacteria than in gram-negative bacteria.

Effect of US duty cycle level on inactivation of *S. aureus*

The inactivation of *S. aureus* populations was found to be dependent on the duty cycle levels ($p<0.05$). According to Fig. 5, initially the inactivation rate of *S. aureus* was increased by extension of the duty cycle to 0.7:0.3, but subsequently it decreased greatly. *S. aureus* has the lowest inactivation at a duty cycle of 0.9:0.1 compared to the two other duty cycles. This phenomenon is probably due to lack of enough time to burst the bubbles, which are made by US waves, in the high duty cycle. Therefore, the lethal effect of US on *S. aureus* decreased in this condition.

Figure 6 shows the effect of duty cycle levels on the inactivation of *S. aureus* during US irradiation. According to the figure, the high inactivation of *S. aureus* was achieved under a duty cycle of 0.7:0.3 s with reduction of the *S. aureus* population to 1.49 CFU/mL.

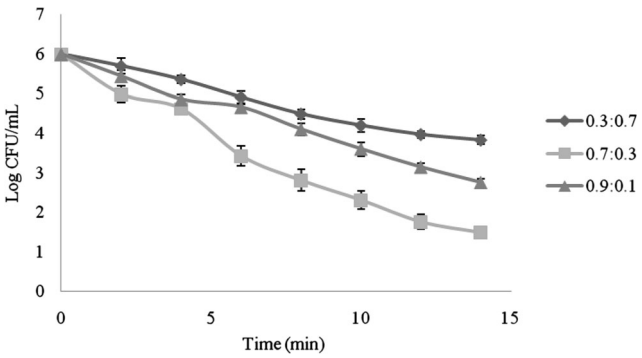

Fig. 6 Effect of duty cycle (on:off time) levels on the inactivation of *S. aureus* during US irradiation

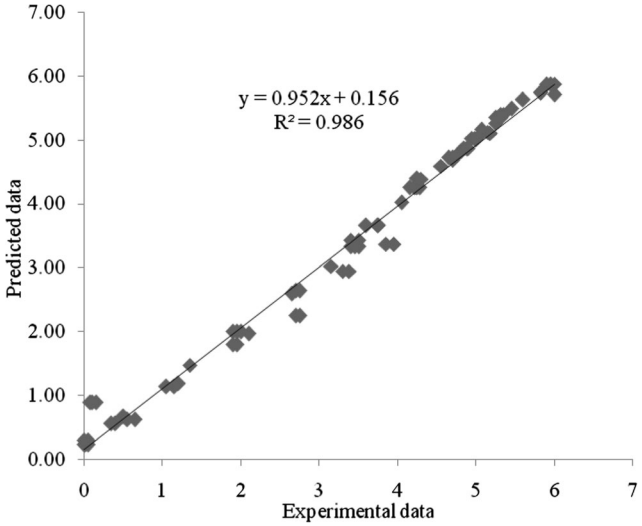

Fig. 7 Experimental versus predicted values of a *S. aureus* population using the GA–ANN model for the test data set (R^2=0.986)

GA-ANN results

GA-ANN model was developed for estimation of the survival of *S. aureus*. In this study, ANN with 2–25 neurons was trained using GA to find the optimal network configuration. It was found that GA-ANN with 17 neurons in one hidden layer could predict a *S. aureus* population with a high coefficient of determination (R^2=0.986). The

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

Hidden neurons	Bias	Input neurons			Output neurons
		Amplitude level (μm)	Duty cycle (s)	Time (min)	*S. aureus* population (CFU/mL)
1	−1.235	1.985	−1.365	0.985	0.895
2	−3.325	0.986	1.325	0.365	0.685
3	0.365	1.325	2.356	−1.365	−1.658
4	−1.125	0.365	−1.362	1.352	0.658
5	0.315	1.236	1.365	0.325	2.325
6	−0.652	0.325	0.225	0.985	1.365
7	1.235	2.315	−0.985	−0.186	−1.356
8	1.635	0.965	1.325	2.152	−0.235
9	−1.325	0.325	1.365	1.023	1.235
10	0.365	2.365	−0.325	−1.865	1.325
11	0.325	0.685	2.365	0.985	−0.658
12	1.365	−1.356	0.685	2.365	0.236
13	−1.356	−0.235	−1.658	0.685	1.365
14	1.895	−0.125	−3.655	−1.658	−1.356
15	−0.325	2.365	0.652	0.685	−1.789
16	2.315	0.685	1.265	0.325	2.325
17	−2.253	0.325	0.685	1.759	−2.356
Bias					−1.256

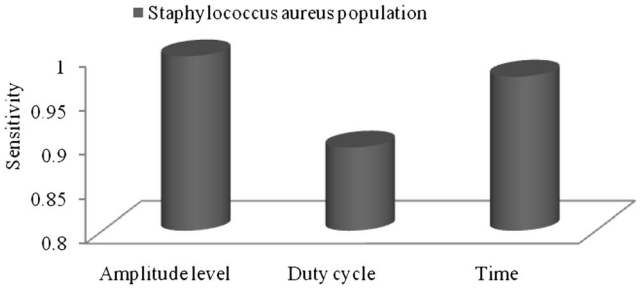

Fig. 8 Sensitivity analysis of optimized GA-ANN (3/17/1) for prediction of *S. aureus*

prediction efficiency of the GA-ANN model for unseen data (testing data) is presented in Fig. 7. The calculated coefficient of determination value for estimation the survival of *S. aureus* 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 the survival of *S. aureus* during US irradiation. The results showed that an acceptable agreement between the predicted and experimental data could be achieved using the GA-ANN model.

Lou and Nakai (2000) 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 to an overall approach for modeling the microbial inactivation dynamics (Cheroutre-Vialette and Lebert 2002). Yolmeh et al. (2014a) used GA-ANN and ANFIS models for prediction of antibacterial activity of annatto dye against *Salmonella enteritidis* and their developed GA-ANN, which included eight hidden neurons, and could predict a *S. enteritidis* population with a correlation coefficient of 0.999.

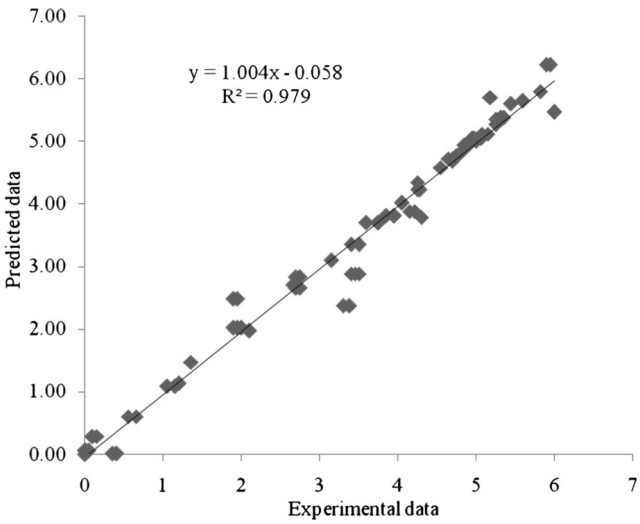

Fig. 9 Experimental versus predicted values of a *S. aureus* population using the ANFIS model for the test data set (R^2=0.979)

Sensitivity analysis was also tested in order to study the sensitiveness of neural network models toward different inputs (Fig. 8). Among the input variables, amplitude level was the most sensitive factor for prediction of *S. aureus* by the selected GA-ANN.

ANFIS results

The ANFIS network parameters, such as the type and number of MF and epochs, have been varied to obtain the best results in terms of model validation. The ANFIS architecture used in this study is shown in Fig. 2. The final ANFIS architecture for predicting the *S. aureus* population, with four Gaussians type MFs for each input (three inputs) and linear MF for output, and the constructed 64 rules resulted in a highly accurate prediction. In Fig. 9 the *S. aureus* 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. aureus* (R^2=0.979). In summary, a lower number of input parameters were needed for the ANFIS model, improving the speed and ease of prediction.

Fernandes et al. (2012) used the ANFIS model to predict antimicrobial peptides activation. They reported that the ANFIS approach could provide an efficient solution for screening putative antimicrobial peptide sequences and for exploration of properties characteristic of antimicrobial peptides.

Conclusions

Alternative methods for pasteurization and sterilization are gaining importance. This is due to increased consumer demand for new methods of food processing that have a reduced impact on nutritional content and overall food quality. US processing is one of the alternative technologies that has shown promise in the food industry. The inactivation of *S. aureus* was found to be dependent on the amplitude and duty cycle levels (p<0.05), and the inactivation rate was increased by an increase of the amplitude levels. The high inactivation of *S. aureus* was achieved under a duty cycle of 0.7:0.3 s with reduction of the *S. aureus* population to 1.49 CFU/mL. It was found that GA-ANN with one hidden layer comprising 17 neurons gives the best fit with the experimental data, which made it possible to predict the *S. aureus* population with acceptable coefficients of determination (0.986). It was also found that ANFIS models with four Gaussian type MFs for all input variables and a linear output give the best fit with the experimental data, which made it possible to predict the *S. aureus* population with a high coefficient of determination (0.979). The results indicated that both GA-

ANN and ANFIS models could give a good prediction of the *S. aureus* population.

References

Adekunte A, Valdramidis VP, Tiwari BK, Slone N, Cullen PJ, Donnell CPO, Scannell A (2010) Resistance of *Cronobacter sakazakii* in reconstituted powdered infant formula during ultrasound at controlled temperatures: a quantitative approach on microbial responses. Int J Food Microbiol 142:53–59

Ananta E, Voigt D, Zenker M, Heinz V, Knorr D (2005) Cellular injuries upon exposure of *Escherichia coli* and *Lactobacillus rhamnosus* to high-intensity ultrasound. J Appl Microbiol 95:271–278

Awad T, Moharram H, Shaltout O, Asker D, Youssef M (2012) Applications of ultrasound in analysis, processing and quality control of food: a review. Food Res Int 48:410–427

Bahramparvar M, Salehi F, Razavi SMA (2014) Predicting total acceptance of ice cream using artificial neural network. J Food Process Preserv 38(3):1080–1088

Bermudez-Aguirre D, Corradini MG, Mawson R, Barbosa-Canovas GV (2009) Modeling the inactivation of *Listeria innocua* in raw whole milk treated under thermo-sonication. Innov Food Sci Emerg 10: 172–178

Chemat F, Khan MK (2011) Applications of ultrasound in food technology: processing, preservation and extraction. Ultrason Sonochem 18:813–835

Cheroutre-Vialette M, Lebert A (2002) Application of recurrent neural network to predict bacterial growth in dynamic conditions. Int J Food Microbiol 73:107–118

Demirdoven A, Baysal T (2008) The use of ultrasound and combined technologies in food preservation. Food Rev Int 25:1–11

Esteve MJ, Frigola A (2007) Refrigerated fruit juices: quality and safety issues. Adv Food Nutr Res 52:103–139

Fernandes FC, Rigden DJ, Franco OL (2012) Prediction of antimicrobial peptides based on the adaptive neuro-fuzzy inference system application. Pept Sci 98(4):280–287

Joyce E, Al-Hashemi A, Mason TJ (2007) Assessing the effect of different ultrasonic frequencies on bacterial viability using flow cytometry. J Appl Microbiol 110:862–870

Kiang WS, Bhat R, Rosma A, Cheng LH (2012) Effects of thermosonication on the fate of *Escherichia coli* O157:H7 and *Salmonella* Enteritidis in mango juice. Lett Appl Microbiol 56: 251–257

Koda S, Miyamoto M, Toma M, Matsuoka T, Maebayashi M (2009) Inactivation of *Escherichia coli* and *Streptococcus mutants* by ultrasound at 500 kHz. Ultrason Sonochem 16:655–659

Lou W, Nakai S (2000) Application of artificial neural networks for predicting the thermal inactivation of bacteria: a combined effect of temperature, pH and water activity. Food Res Int 34:573–579

Mason TJ (2007) Sonochemistry and the environment – providing a "green" link between chemistry, physics and engineering. Ultrason Sonochem 14:476–483

Oonmetta-aree J, Suzuki T, Gasaluck P, Eumkeb G (2006) Antimicrobial properties and action of galangal (*Alpinia galanga* Linn.) on *Staphylococcus aureus*. Food Sci Technol 39(10):1214–1220

Patil S, Bourke P, Kelly B, Frias JM, Cullen PJ (2009) The effects of acid adaptation on *Escherichia coli* inactivation using power ultrasound. Innov Food Sci Emerg 10:486–490

Piyasena P, Mohareb E, McKeller RC (2003) Inactivation of microbes using ultrasound: a review. Int J Food Microbiol 87:207–216

Raso J, Pagan R, Condan S, Sala FJ (1998) Influence of temperature and pressure on the lethality of ultrasound. Appl Environ Microbiol 64(2):465–471

Rodgers SL, Ryser ET (2004) Reduction of microbial pathogens during apple cider production using sodium hypochlorite, copper ion, and sonication. J Food Prot 67:766–771

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

Sango DM, Abela D, McElhatton A, Valdramidis VP (2014) Assisted ultrasound applications for the production of safe foods. J Appl Microbiol 116:1067–1083

Soleimanzadeh B, Hemati L, Yolmeh M, Salehi F (2014) GA-ANN and ANFIS models and *Salmonella enteritidis* inactivation by ultrasound. J Food Saf. doi:10.1111/jfs.12174

Tsukamoto I, Yim B, Stavarache CE, Furuta M, Hashiba K, Maeda Y (2004) Inactivation of *Saccharomyces cerevisiae* by ultrasonic irradiation. Ultrason Sonochem 11:61–65

Ugarte-Romero E, Feng H, Martin SE (2007) Inactivation of *Shigella boydii* 18 IDPH and *Listeria monocytogenes* Scott A with power ultrasound at different acoustic energy densities and temperatures. J Food Sci 74(4):103–107

Yolmeh M, Habibi-Najafi MB, Salehi F (2014a) Genetic algorithm-artificial neural network and adaptive neuro-fuzzy inference system modeling of antibacterial activity of annatto dye on *Salmonella enteritidis*. Microb Pathog 67–68:36–40

Yolmeh M, Habibi-Najafi MB, Farhoosh R, Salehi F (2014b) Modeling of antibacterial activity of annatto dye on *Escherichia coli* in mayonnaise. Food Biosci 8:8–13