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Modeling of antibacterial activity of annatto dye on *Escherichia coli* in mayonnaise

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ABSTRACT

Annatto ranks second in economic importance worldwide among all natural colorants and its extract fraught with antimicrobial and antioxidant properties. In the present paper, adaptive neuro-fuzzy inference system (ANFIS) and genetic algorithm–artificial neural network (GA–ANN) models were undertaken to predict the annatto dye on *Escherichia coli* in mayonnaise. The ANFIS and GA–ANN 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 (1–17 days) for prediction of *E. coli* population. Both models were trained with experimental data. The results revealed that the annatto dye was able to decline *E. coli* and the bactericidal effect of annatto dye was stronger at 25 °C than that in 4 °C. The developed GA–ANN, included 13 hidden neurons, could predict *E. coli* population with coefficient of determination of 0.995. The largely agreement between experimental and ANFIS predictions data was also acceptable (R^2=0.991). Sensitivity analysis results revealed that storage time was the most sensitive factor for prediction of *E. coli* population.

1. Introduction

Mayonnaise is one of the oldest sauces widely consumed around the world. Preservatives are being added to mayonnaise due to the insufficient heat treatment in the processing steps. Using natural alternatives instead of synthetic preservatives is promising because synthetic preservatives are believed non-safe and potentially harmful (Da Silva & Franco, 2012).

Escherichia coli serves as food-borne pathogens (Altkruse, Cohen, & Swerdlow, 1997). It can break out by mayonnaise. In March 1993, *E. coli* outbreak happened with 50 cases in Oregon of US through consumption of mayonnaise (Hathcox, Beuchat, & Doyle, 1995). Colonization of microorganisms in mayonnaise varies on the pH, type of acid used, temperature and storage time.

Annatto dye is characterized by properties such as antimicrobial, anticancer, and antioxidant activity (Kurniawati, Soetjipto, & Limantara, 2007; Prabhakara Rao, Satyanarayana, & Rao, 2002). The antimicrobial activity of annatto dye is due to several mono and sesquiterpenes (Magiatis, Melliou, Skaltsounis, Chinou, & Mitaku, 1999). Annatto dye is widely prepared in the food preparation, exhibiting antimicrobial activity.

Da Silva and Franco (2012) investigated the using of oregano essential oil against *Salmonella enteritidis* in mayonnaise salad;

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they found that number of S. *enteritidis* decreased by oregano essential oil in mayonnaise.

In spite of enormous reports in literature on kinetics of microbial thermal, to the best of our knowledge, there has been relatively little data reported on quantitative data of non-thermal inactivation. In the former case, the rate of bacterial inactivation has usually been calculated using linear regression (log number survivors versus time). Anyway, in recent times it has been revealed that many bacterial inactivation curves do not follow linear manner (Koutsoumanis, Lambropoulou, & Nychas, 1999).

The non-linear methods of artificial neural networks (ANN) and adaptive neuro-fuzzy inference system (ANFIS) lie in the black box modeling group. Researchers examined the potential of ANN and ANFIS as an analytical alternative to conventional modeling techniques, which are frequently limited by strict assumptions of normality, linearity, homogeneity, and variable independence (Mashrei, Abdulrazzaq, Abdalla, & Rahman, 2010; Rumelhart, Durbin, Golden, & Chauvin, 1994). Fuzzy inference systems (FIS) and ANNs are model-free numerical estimators. To be used in optimized manner, FISs and ANNs could be combined into an integrated system called ANFIS; the integrated system then has the utility 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) (Mashrei et al., 2010).

Neuro-fuzzy employs neural network learning functions to refine each part of the fuzzy knowledge separately. Learning in a separated network is much more rapid than learning in an entire network. One approach to the derivation of a fuzzy rule base is to practice the self-learning features of artificial neural networks, to explain the membership function based on input–output data (Ghoush, Al-Mahasneh, Samhouri, Al-Holy, & Herald, 2009). The determination of membership function parameters and fuzzy rules is not easy for problems that are more complex. ANFIS structure gives an easy way to generate the membership functions and fuzzy rules for surgeon type fuzzy inference systems (Gulbag & Temurtas, 2006).

Lou and Nakai (2000) performed an ANN to evaluate the effects of pH, temperature, and a_w on the thermal inactivation rate of *E. coli*. The methodology gave accurate results compared to other secondary models. Besides, the performance of ANNs as an integrated primary–secondary inactivation model can contribute in an overall approach for modeling the microbial inactivation dynamics (Cheroutre-Vialette & Lebert, 2002).

There is no study available in the literature concerning the use of computing technology for prediction of the annatto dye on *E. coli*. Therefore, the present research is aimed to investigate the effect of annatto dye concentration, storage temperature and storage time on *E. coli* population and study the performance of GA–ANN and ANFIS to the microbial inactivation modeling.

2. Materials and methods

2.1. *Material*

Organic solvents and mediums used were all analytical grades and provided from Merck, Germany. Annatto seeds were prepared from the local market of Hyderabad, India. *E. coli* ATCC 25922 acquired from the Department of Food Science and Technology, Ferdowsi University of Mashhad.

2.2. *Dye extraction*

Annatto seeds were drenched in n-hexane for 6 h to eliminate oils, and then dye was extracted by acetone from defatted seeds (Castello, Chandra, Phatak, & Madhuri, 2004). The extract was concentrated by the rotary evaporator after filtration through Whatman filter paper no. 1 and afterwards vacuum-dried in a vacuum oven model 1410D-2E (Shel Lab, USA) to yield dye powder.

2.3. *Mayonnaise preparation*

Mayonnaise was prepared in the laboratory, 4% acetic acid and yolk were used as emulsifier and as an acidic agent, respectively. The powder of annatto dye was supplemented to mayonnaise formulation with final concentration of 0.1%, 0.2% and 0.4% (v/v). pH of the produced mayonnaise was measured 4.4. The bacterial suspension was inoculated to each sample to acquire ultimate concentration of 1.5×10^8 cfu/g. All samples were stored at refrigerator (4 °C) or room (25 °C) temperature.

2.4. *Determination of antimicrobial activity*

10 g mayonnaise was dissolved in 90 ml of ringer and a series of 10-fold dilutions was obtained from this suspension and then performed for plating on plate count agar (Da Silva & Franco, 2012). The mayonnaise samples stored 17 days, due to survival of the bacteria reached to the minimum of population after 17 days at 4 °C.

2.5. *GA–ANN model*

The well-liked ANN is the multi-layer feed-forward neural network, whose neurons are organized into three layers of input, hidden and output. A diagrammatic depiction 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 is related to the number of input variables into the neural network, and the number of output neurons is same to the number of target output variables. Between the input and the output layers, there is at least one hidden layer varied with any number of neurons and the application of the network. Definition of

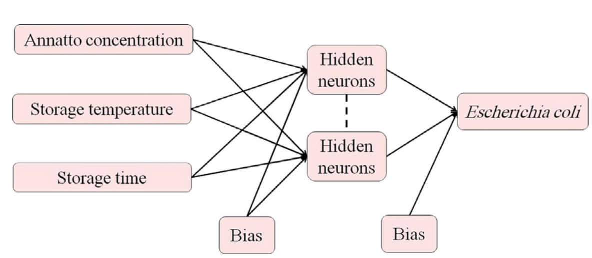

Fig. 1 – ANN architecture with one hidden layer for prediction of *E. coli* population.

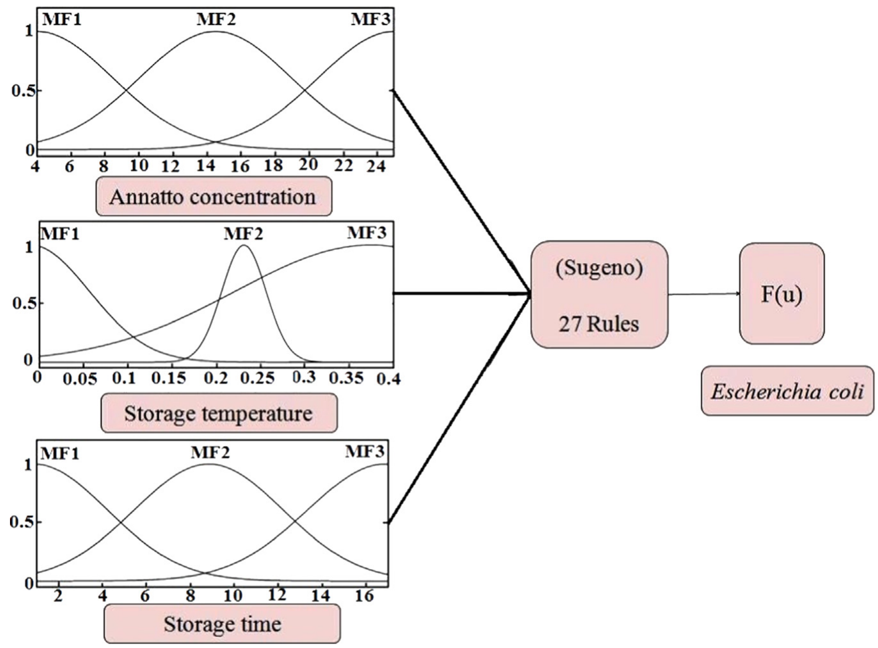

Fig. 2 – The general structure of ANFIS for the *E. coli* population model with 3 inputs.

optimum number of hidden layer neurons is usually carried out according to trial and error method (BahramParvar, Salehi, & Razavi, 2013; Salehi & Razavi, 2012). Genetic algorithm (GA) optimization technique can be used to overcome this intrinsic limitation of ANN. GA are known to be search techniques for an optimal value, mimicking the mechanism of biological development. They have a high potential to find an optimal value (global optimal value or at least near global one) of a complex purpose function, without falling into local optima (Morimoto, 2006; Salehi & Razavi, 2011).

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 represents the inputs, w_{ij} is the weight associated with each input/node connection, n is the number of nodes and b_j denotes the bias associated with node j. Moreover, bias is an extra input added to neurons. Because of this adding, the bias term allows a representation of phenomena having thresholds (Salehi & Razavi, 2011). A sigmoid activation function (Eq. (2)) was chosen to be used as the transfer function in the hidden and output layers:

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

Two hundred and fifty results were obtained from experiments in the present study, and then all data were randomly parted into 3 partitions: training (25%); validating (25%); and testing data (50%). The testing data was used for appraising the act 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 at 0.9 and 0.01, respectively.

Sensitivity analysis was conducted to provide an evaluation of the relative importance among the inputs of the neural network model and to demonstrate how the model output diverse in response to variation of an input (BahramParvar et al., 2013). In the present study, the Neurosolution software (release 6.01, Neuro Dimension, Inc., USA) was performed to design the GA–ANN model.

2.6. *ANFIS model*

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 acquiring a data set (input–output data points). The data order was first randomized and then all data were divided into three sections: 25%; 25%; and 50% of total data was used to training, validating and testing (unseen data) the network, respectively. Each input/output pair contained three inputs (the annatto dye concentration, storage temperature and time) and one output (*E. coli* population) (Fig. 2). The number of membership functions allocated 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 prediction of the *E. coli* population.

3. Results and discussion

3.1. *Effect of annatto dye on E. coli*

Fig. 3 shows the effect of different concentrations of annatto dye on *E. coli*. It is obvious that as the annatto extract concentration in mayonnaise increases, the viability of *E. coli* in mayonnaise decreases compared to the control significantly.

Fig. 4 indicates the variation in populations of *E. coli* stored under refrigeration and ambient temperature. In the figure, number of CFU is mean of CFUs at different concentration of

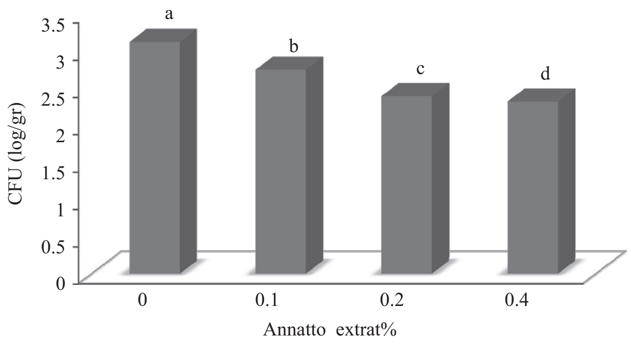

Fig. 3 – Effect of annatto dye on *E. coli* growth in the mayonnaise.

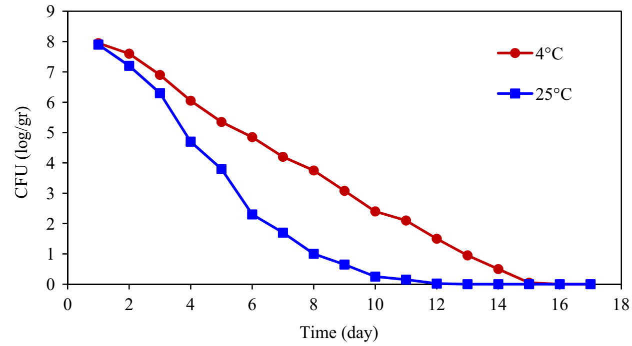

Fig. 4 – The effect of temperature on *E. coli* in the mayonnaise contains annatto dye during storage.

annatto dye (0%, 0.1%, 0.2% and 0.4%) at each temperature. The mean was calculated by Minitab® version 16.1.1 (Minitab Inc. USA. 2010). The population of *E. coli* declined at both 4 and 25 °C during storage. This reduction was higher in 25 °C compared to 4 °C. No *E. coli* was found after 12 days of and 15 days inoculation at 25 and 4 °C, respectively. Our finding is in agreement with other authors' e.g. Hathcox et al. (1995). They observed that death rate of *E. coli* intensified in mayonnaise by increasing storage temperature from 5 to 30 °C (Hathcox et al., 1995). This might be due to changes in the bacterial cell membrane at 4 °C in such a way to reducing penetration of antimicrobial compound in to the cell (Smith-Palmer, Stewart, & Fyfe, 1998).

Heo et al. (2010), evaluated inactivation of *Salmonella typhimurium* in commercial mayonnaise at various temperatures and pH, and concluded that also most declines occurred in the viability of *S. typhimurium* in 40 °C than 10 °C. Lock and Board (1994) achieved the same results while evaluating viability of *S. enteritidis* in different mayonnaise.

3.2. GA–ANN

GA–ANN model was performed for assessment of *E. coli* population. 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 13 neurons in one hidden layer could predict *E. coli* population with high coefficient of determination (R^2=0.995). The prediction efficiency of the GA–ANN model for unseen data is shown in Fig. 5. The calculated coefficient of determination value for estimation of *E. coli* population shows strong correlation between predicted and experimental values. Table 1 demonstrates the weights and bias values of optimized network, which could be applied in a computer program for estimation of *E. coli* population in mayonnaise. The results revealed that an acceptable agreement between the predicted and experimental data could be achieved using GA–ANN model. Lou and Nakai (2000) reported that ANN was used successfully to predict the thermal inactivation of *E. coli*.

Sensitivity analysis was tested in order to evaluation the sensitiveness of neural network models toward different inputs (Fig. 6). Among the input variables, storage time was the most sensitive factor, followed by storage temperature and eventually annatto concentration for prediction of *E. coli*

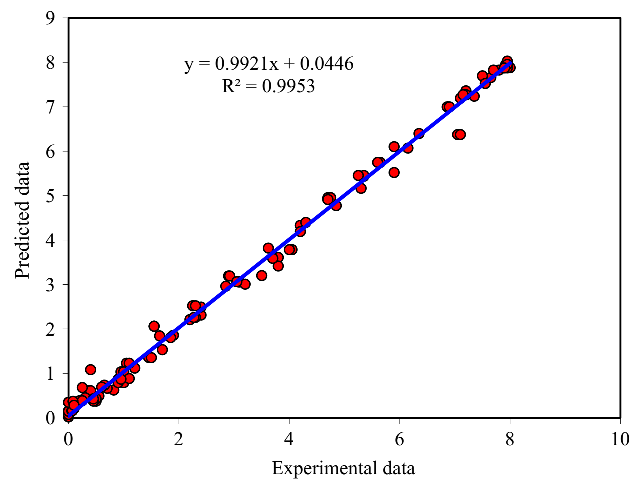

Fig. 5 – Predicted versus experimental values of *E. coli* population using GA–ANN model.

population by the selected GA–ANN. However Yolmeh, Habibi Najafi, and Salehi (2014) observed that the storage temperature was the most sensitive factor among the parameters for prediction of *S. enteritidis* population by the selected GA–ANN.

3.3. ANFIS

The ANFIS network factor, such as the type and number of membership function and epochs, have been diverse to acquire the best results in terms of model validation. ANFIS architecture used in this study is shown in Fig. 2. The final ANFIS architecture for predicting the *E. coli* population, with three Gaussians type membership functions for each input (3 inputs) and linear membership function for output was applied and constructed 27 rules, resulting in high precise prediction. In Fig. 7, the *E. coli* population 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 *E. coli* (r=0.991).

4. Conclusion

Annatto is commonly used as a coloring agent in food industry with antimicrobial and antioxidant properties. The

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	*Escherichia coli* population
1	0.152	1.652	−1.635	0.635	1.325
2	−0.023	1.253	0.235	0.685	1.759
3	−1.325	1.325	1.356	−1.235	2.658
4	1.325	0.365	1.365	1.352	0.658
5	1.256	1.236	1.365	0.325	1.325
6	0.365	0.652	0.365	0.325	1.256
7	1.235	2.365	0.985	0.986	−2.365
8	1.153	0.235	0.685	1.759	1.356
9	−0.365	1.365	1.352	0.352	1.325
10	1.236	1.256	0.325	0.325	0.365
11	0.325	0.365	0.985	1.365	1.526
12	−0.385	1.236	1.365	0.325	0.325
13	1.325	0.365	0.685	1.325	0.365
Bias					0.325

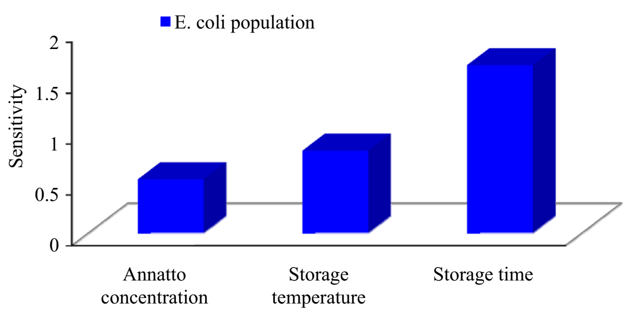

Fig. 6 – Sensitivity analysis of optimized GA–ANN (3/13/1) for prediction of *E. coli* population in the mayonnaise.

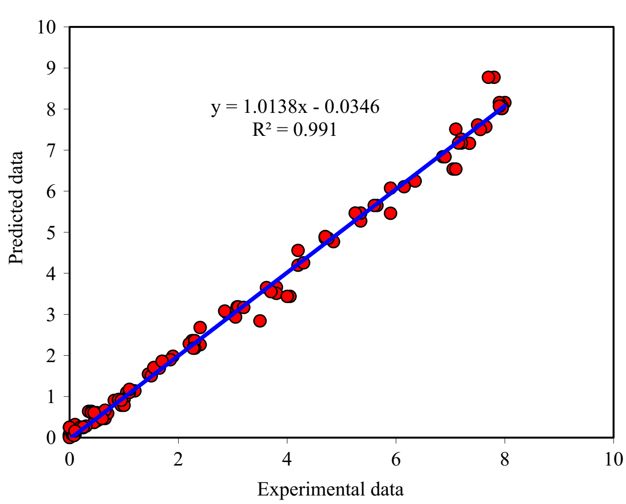

Fig. 7 – Predicted versus experimental values of *E. coli* population using ANFIS model.

survival curve of *E. coli* in mayonnaise reached to zero during 15 and 12 days after inoculation at 4 and 25 °C, respectively. Giving to the results, thanks to its antimicrobial activity, annatto dye can be suggested to be performed as an alternative to synthetic preservatives in the formulation of mayonnaise. GA–ANN with 1 hidden layer comprising 13 neurons gives the best fitting with the experimental data, which made it possible to predict *E. coli* population with an acceptable coefficient of determination (0.995). It was also observed that ANFIS models with three Gaussian type membership functions for all input variables and linear for output gives the best fitting with the experimental data, allowing the prediction of *E. coli* population with high coefficient of determination (0.991).

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