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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

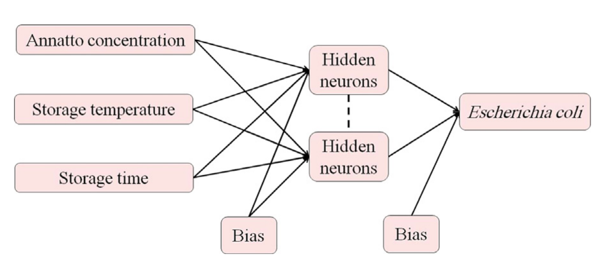

Fig. 1 – ANN architecture with one hidden layer for prediction of *E. coli* population.