MODELING OF CURED MEAT COLOUR FORMATION USING GENETIC ALGORITHM AND ARTIFICIAL NEURAL NETWORK

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Abstract – In this study the yield of cured meat pigment formation and its colour characteristics in model system of heme were evaluated at five different concentrations of HCl, nitrite and ascorbic acid. A feed-forward neural network was used to estimate the yield, wavelength of maximum absorbance (λ_{max}), ΔL^*, Δa^* and Δb^* of nitrosoheme produced during curing process. The results were compared with experimental data and a high correlation between the experimental and predicted values was found., The process conditions were then optimized for the maximum yield and Δa^*, minimum ΔL^* and Δb^*, and λ_{max} equal to 540 nm using genetic algorithm. The best fitness was achieved by HCl concentration of 0.58% and nitrite and ascorbic acid concentration of 406.14 and 388.75 mM respectively for conversion of 1mM hemin to nitrosoheme.

Key Words – Artificial Neural Network; Genetic Algorithm; Nitrosoheme.

I. INTRODUCTION

One of the most important characteristics of raw or cured meat is its colour. The distinguished colour of raw cured meat (i.e., before thermal processing) is due to NOMb that is produced by reaction of nitric oxide (NO) with central iron of heme in myoglobin. After thermal processing, globin denatures, detaches itself from the iron atom and surrounds the heme moiety. Although the effect of nitrite and reductants on formation of cured meat colour has been the subject of several studies [1], this process is still not modeled.

One of the computational modeling tools is Artificial Neural Network (ANN) that has recently emerged and found extensive acceptance in many disciplines for modeling real-world problems. ANN may be defined as structures consisted of densely interconnected adaptive simple processing elements (called artificial neurons or nodes) which are capable of performing massively parallel computations for data processing and knowledge representation [2]. ANN is a rather versatile technique, therefore, it is not surprising that ANN has found applications in different realms of food [3]. For example ANN has been used for modeling of food extrusion process [4], processing of sensor data , identification of different strains of bacterial contaminants[5], prediction of mass transfer kinetics [6] and sensory evaluation [7].

Genetic algorithms (GAs) are powerful optimization techniques based on the methods of evolution. The optimization method of genetic algorithm includes the generation of possible solutions, application of selection, crossover and mutation operations and evaluation of each solution over an objective function (fitness function) until a certain stopping criterion is met .

In this study, in the first step, the yield of nitrosoheme formation and its colour in model system under different HCl, nitrite, and ascorbic acid concentrations were investigated by artificial neural network. Then, the best conditions for formation of cured colour were achieved using genetic algorithm.

II. MATERIALS AND METHODS

Extracted heme from meat was used as a model system for prediction of cured meat colour formation. Extraction of heme was done according to the procedure of Hornsey (1956) [8]. Minced lean meat was defatted by hexane cold extraction and homogenized with the mixture of acetone: water: HCl. For each 100 g of meat, 80 ml acetone and 3 ml water was used. Water was replaced by the amount of concentrated HCl mentioned in experimental design.

For preparation of nitrosoheme, nitrite and ascorbic acid were added in proper concentrations to each 25 ml of the extracted heme. The amount of nitrite and ascorbic acid was determined for each 1 mM of heme. The container was gently shaken until nitric oxide slowly bubbled into the mixture and emitted into the air from the top of container. The container was then capped and shaken vigorously for 30 s. The preformed, cooked cured-meat pigment was stored in the dark until further application. The yield of nitrosoheme pigment was determined according to Hornsey (1956) [8].

The absorption spectrum of nitrosoheme pigment was recorded using a Camspect spectrophotometer. The lightness/darkness (L^* value), red/green (+/- a^* value), and yellow/blue (+/- b^* value) of the nitrosoheme pigment was determined according to Yam and Papadakis (2004) [9].

The experiments were conducted as a 5×5×5 factorial design (5 HCl concentrations × 5 nitrite concentrations × 5 ascorbic acid concentrations). The HCl concentrations were 0.4, 0.6, 0.8, 1 and 1.2 %. Nitrite was used in five levels of 100, 200, 300, 400 and 500 mM when Ascorbic acid concentrations were 50, 150, 250,350 and 450 mM. For the prediction of yield and colour of nitrosoheme production, fully interconnected multilayer structure (MLP) of feed-forward neural network (FFNN) with perceptron training rule, which is the most widely used ANN, was applied. The complexity of the MLP network depends on the number of layers and the number of neurons in each layer. Mathematically:

$$y_j = \sum_{i=1}^{n} f\left(w_{ij} x_i\right) + b_j \quad (1)$$

where y_j is the output of a neuron, Wij is the weight of the ith input vector that is connected to the jth neuron; n is the number of inputs to the neuron; bj is the bias associated with the jth neuron, which adds a constant term in the weighted sum to improve convergence; and f is the activation function that determines the processing inside the neuron. In this work, the operational variables of nitrosoheme formation (HCl, nitrite and ascorbic acid concentration) were used as inputs, and for each one of yield, wavelength of maximum absorbance, ΔL^*, Δa^* and Δb^*, a separate ANN was developed. Hyperbolic tangent, linear and logarithmic sigmoid activation functions were chosen to be used in the hidden layer. ANN with three layers was used in this research, except for Δb^* that a quadratic-layered ANN was applied.

From the 125 data, the incorrect responses were deleted, and in total, 108 data were collected for three different concentrations of nitrite, ascorbic acid, and HCl. To improve the training performance, input and output variables were normalized, i.e., each variable was mapped into the range of [-1 +1]. Next, the data were randomly divided into three partitions. The first partition (training data) was used to perform the training of the network (60% of data). The second one (validation data) was used to evaluate the prediction quality of the network during the training (20% of data). For the purpose of estimating the performance of the trained network on new data, a third partition, which was never seen by the artificial neural network during the training, was used (20% of data) for testing. Levenberg-Marquardt back propagation optimization was utilized for updating weight and bias states. The training process was carried on for 2,000 epochs, or until the validation data's mean-squared error (MSE), calculated by Eq. 2, did not improve for 1000 epochs to avoid over-fitting of the network.

The predictive power of a theoretical model was determined by several statistics. In addition to the correlation coefficient (r^2) between the observed and predicted values, the MSE was computed as:

$$MSE = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 / n = \sum_{i=1}^{n} \Delta_i^2 / n, \quad (2)$$

where y_i and $\hat{y}_i$ are the observed and predicted values for the object i, respectively, Δi is the corresponding residual, and n is the number of samples. In addition, normalized mean-squared error (NMSE) and the mean of absolute error (MAE) were also calculated by

$$NMSE = \frac{1}{\sigma^2 n} \sum_{i=1}^{n} \Delta_i^2, \quad (3)$$

$$MAE = \frac{1}{n} \sum_{i=1}^{n} |\Delta_i| \quad (4)$$

Genetic algorithm which is based on the principle of a Darwinian-type survival of the fittest in natural evolution is essentially an iterative, population-based, parallel global search algorithm that has a high ability to find optimal value of a complex objective function, without falling into local optima. In this study, the ranges for the control variables were set and an initial population of 100 chromosomes was randomly created. Among the 50 individuals in each generation, crossover rate was set to 80%, while 2 elites were selected to be copied in the next generation. The individual chromosomes in the genetic algorithm population were evaluated using the developed FFNN prediction model. The FFNN models predicted the yield and colour of nitrosoheme under the defined constraints. The fitness of a chromosome in the population was evaluated over minimizing the following objective function:

$$f = \{25-(2\times y_a) + y_L + y_b - (10\times y_{yn})\}*\lambda_{max} \quad (5)$$

where, y_a, y_L, y_b and y_{yn} are predicted Δa^*, ΔL^*, Δb^* and yield of nitrosoheme formation using the FFNN. Since there were only two wavelengths of 510 and 540 nm for maximum absorption, and 540 nm is the optimum wavelength for the maximum absorbance of nitrosoheme pigment, this variable was fitted as a constrain for the fitness function. λ_{max} was defined as 1 for wavelength of 540 nm, and a large number for wavelength of 510 nm, penalizing the individuals with this wavelength and preventing their growth. The coefficients in Eq. 16 indicate the importance of each variable in determining the optimal point. The genetic algorithm stopping criterion was set as evolution of 200 generations.

III. RESULTS AND DISCUSSION

Results indicated that with simultaneous increases in nitrite and ascorbic acid concentrations, the yield of nitrosoheme production is enhanced, however, in high levels of nitrite and ascorbic acid a slight decrease in the yield was observed. When nitrite and ascorbic acid are present at enough concentrations, nitric oxide is produced in adequate quantities to transform all hemin to nitrosoheme. In higher concentration of these two compounds, the structure of heme is damaged and cured colour is changed to other pigments. The concentration of HCl influences the yield more than two other variables so that at different concentrations of HCl, the yield changes slightly with the increase in ascorbic acid and nitrite concentrations. It seems that HCl has two effects on nitrosoheme synthesis: it decreases pH and facilitates reactions that transform nitrite to nitric oxide. As the reactivity of the nitrite/nitrous acid system increases with decreasing pH, the concentration of nitric oxide is maximized, and the yield increases to 100%. Also, in low concentrations of HCl, chloride ions play a role in nitrosating reactions. Basheer & Hajmeer (2000), examining the importance of sodium chloride in meat curing, concluded that chloride ions could actually help catalyse nitrosation reactions [10].

At lower concentrations of HCl and higher concentrations of ascorbic acid, nitrosoheme production increases, accompanied by a higher a^* value. By increasing HCl concentrations, the produced pigment decomposes, leading to a decrease in redness. The greater redness of the pigments with sodium nitrite is due to the fact that it is reduced to nitric oxide by added reductant (i.e., ascorbate) and reacts with hemin to form the red nitrosoheme. Although the a^* values have not been evaluated in synthesized pigments, the effect of higher levels of nitrite on increasing the redness of meat products has been confirmed [11]. The chloride ion of HCl at low concentrations influences the production of pigments with higher a^* values, and increasing the concentration of HCl, or in other strongly acidic conditions, nitric oxide dissociates after production of nitrosoheme, a phenomenon like light fading that decreases the a^* value and produces a pigment with green colour.

With increases in HCl concentration and reductions in nitrite and ascorbic acid concentrations, lightness is enhanced. At low concentrations of HCl, the L^* value reduced with increases in ascorbic acid concentrations. Simultaneous increases in nitrite and ascorbic acid concentrations cause the L^* value to decrease. When the concentration of ascorbic acid or nitrite decreases, the b^* value increases, and HCl at low or high concentrations decreases this value. In these conditions, cured-meat pigments form, and they dissociate at high HCl concentrations after production and convert to oxidized heme.

Statistical assessments of the models in the training set, test set, and validation set, namely r^2, MSE, NMSE, MAE are listed in Table 1. The small values of errors indicate that the models are highly accurate. In addition, the fulfillment of all validation criteria in three sets of data also warrants its predictivity. Statically, the high values of r^2 given rise by the models signify the fact that these theoretical models exhibited highly statistical significance between the theoretical model and the input data.

The individual chromosomes representing the combinations of three control variables in the genetic algorithm population were evaluated using the FFNN network to predict yield of nitrosoheme production and colour parameters in the final product. The optimum conditions for the curing colour development were evaluated over the fitness function given in Eq. 5. The optimal process conditions for the best fitness are HCl concentration of 0.58% and nitrite and ascorbic acid concentration of 406.14 and 388.75 mM for conversion of 1mM hemin to nitrosoheme. In these conditions, the predicted responses are yield=99.74%, Δa^*=12.80, ΔL^*= -5.44, Δb^*=-3.67 and λ_{max}=540nm that are in agreement with experimental values.

Table 1. Errors in prediction of nitrosoheme formation using ANN.

	R^2	MSE	NMSE	MAE
Test				
Yield	0.983	1.0458	1.1242	0.8228
λ_{max}	1	2.4×10^{-9}	0.973	1.62×10^{-5}
ΔL^*	0.957	0.0862	0.9549	0.2317
Δa^*	0.987	0.3237	0.9583	0.3102
Δb^*	0.982	0.0968	1.0201	0.1712
Validate				
Yield	0.978	1.0499	0.9574	0.8286
λ_{max}	1	7.19×10^{-11}	0.990	7.19×10^{-6}
ΔL^*	0.976	0.0650	1.1422	0.2092
Δa^*	0.987	0.3476	0.9846	0.4511
Δb^*	0.984	0.056	1.0333	0.15251
Train				
Yield	0.969	1.7452	0.9705	1.015
λ_{max}	1	3.2×10^{-10}	0.975	1.03×10^{-5}
ΔL^*	0.950	0.0839	0.9688	0.2274
Δa^*	0.982	0.5199	0.9803	0.4467
Δb^*	0.985	0.0865	0.9829	0.1277

IV. CONCLUSION

Results indicate that HCl concentration has a major effect on yield and colour of nitrosoheme. In lower concentrations of HCl, yield of nitrosoheme formation and its redness increases. Nitrite and ascorbic acid concentration have a nonlinear effect on cured meat colour formation. With increasing nitrite and ascorbic acid concentrations, cured meat colour formation enhances, whereas it decreases at high levels of the compounds. Feed-forward neural network can accurately predict the results and good fitness of predicted and experimental values was shown with high r^2 (0.96-1) and low MSE, NMSE and MAE. Genetic algorithm predicted the optimum conditions for cured meat colour formation at HCl, nitrite, and ascorbic acid concentration of 0.58%, 406.14 mM and 388.75 mM, respectively for conversion of 1mM hemin to nitrosoheme.

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