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Estimation of peroxidase activity in red cabbage by artificial neural network

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RESEARCH PAPER

Abstract

Enzymes in plant tissues can have undesirable or desirable effects on the quality of fruits and vegetables such as the post-harvest senescence, oxidation of phenolic substances, starch-sugar conversion and post-harvest demethylation of pectic substances leading to softening of plant tissues during ripening. Peroxidase (POD) is a commonly enzyme in vegetable, with bad effects on quality of their products. Structure of POD and POD isoform differ in each vegetable. Hence, activity of POD is unique to each vegetable. The aim of this study was evaluation efficiency of different essential oils as natural antioxidant in POD inactivation and estimation of POD activity by artificial neural network (ANN) modeling. In this study we used natural antioxidant (cumin, fennel, clove) in red cabbage. An ANN was developed by using a multilayer perceptron model, three input neurons (type and concentration of antioxidant and duration of enzyme activity), one hidden layer and 21 hidden neurons. The ANN model predicted POD activity with a mean square error of 0.0002629 and a good correlation between predicted and experimental data (R^2=0.9974). These results show the ability of ANN technology for predicting POD activity of red cabbage under natural antioxidants.

Keywords: artificial neural network, natural antioxidant, peroxidase

1. Introduction

Enzymes in plant tissues can have undesirable or desirable effects on the quality of fruits and vegetables such as the post-harvest senescence, oxidation of phenolic substances, starch-sugar conversion and post-harvest demethylation of pectic substances leading to softening of plant tissues during ripening. The major factors which are mostly used to control enzyme activities are temperature, water activity, pH and the use of certain chemicals during processing (Rui *et al.*, 2006).

Peroxidase (POD) is an enzyme commonly found in vegetables which binds to hydrogen peroxide and produces an activated complex that can react with a wide range of donor molecules and cause off-flavours and colours in raw and un-blanched frozen vegetables (Aruoma *et al.*, 1996; Lee and Klein, 1989). Inhibition of the enzyme activity in fruits and vegetables is generally achieved using physical or chemical treatments such as heating (blanching), lowering pH and/or water activity or adding chemical additives. Several studies to extend shelf-life of minimally processed fruits and vegetables have focused on methods such as using acidulants, reducing and chelating agents, and inorganic salts. However, due to the market demands of the consumer, which is concerned about the use of chemicals in such products, more attention has been given to the search for alternative anti-browning compounds (Daraei Garmakhany *et al.*, 2010; Gunes and Bayindirli, 1993).

In food technology, artificial neural networks (ANN) were applied to predict rheological properties of dough (Razmi-Rad *et al.*, 2007), image analysis of dried fruit (Fathi *et al.*, 2009) and food quality control (Kashaninejad *et al.*, 2009). And in bioprocessing, neural networks were applied as sensors for enzyme engineering (Linko *et al.*, 1999), thermal inactivation of glucoamylase (Bryjak *et al.*, 2004) and estimation of kinetic parameters in enzymatic reaction (Baş and Boyacı, 2007; Baş *et al.*, 2007a,b). Mathematical models are very useful for the design and optimization of many biochemical processes. This method is sometimes difficult to realize since the created models, based on microbial or enzyme kinetics, are very often highly complex. On the other hand, the obtained model leads to a better understanding of the process and is reliable for interpolation and extrapolation (Bryjak *et al.*, 2004).

The amount of covalently bounded carbohydrates significantly differs for POD isoforms or POD from different sources (Yang *et al.*, 1996). Hence, investigating the activity of POD requires knowledge of critical factors such as enzyme inactivation kinetic parameters which is unique to each vegetable (Rudra Shalini *et al.*, 2008).

Artificial neural network

Neural networks are universal approximators that possess the ability to approximate any real-value continuous function to any desired degree of accuracy. They require relatively little time to construct and do not require any prior knowledge of the relationships between the process variables in question and can, therefore, be considered to be 'black box' systems (Aghajani *et al.*, 2012; Kashiri *et al.*, 2012).

One of the commonly used feed-forward ANN architectures in food technology is the multilayer perceptron (MLP) network (Equation 1) (Aghajani *et al.*, 2012; Fathi *et al.*, 2009; Kashaninejad *et al.*, 2009; Kashiri *et al.*, 2012; Razmi-Rad *et al.*, 2007). MLP consists of (a) an input layer with neurons representing input variables to the problem; (b) an output layer with neuron(s) representing the dependent variable(s); and (c) one or more hidden layers containing neuron(s) to help capture the nonlinearity in the system.

$$y_i = \sum_{i=1}^{i} x_i w_{ij} + b_j \tag{1}$$

Where y_i are the net inputs to node j in the hidden or output layer, x_i are the inputs to node i (or outputs of previous layer), w_{ij} are the weights representing the strength of the connection between the i^{th} node and j^{th} node, i is the number of nodes and b_j is the bias associated with node j.

The accuracy of the MLP network for predicting depends on the number of layers, number of neurons in each layer, transfer function, number of epoch, learning rate and momentum (Fathi *et al.*, 2009; Razmi-Rad *et al.*, 2007). The optimal conditions were selected by using a trial and error method. The activation function can be a linear or nonlinear function depending on the network topology.

The hyperbolic tangent (tanh) and linear sigmoid are two common functions. The tanh applies a bias and tanh function (Equation 2) to each neuron in the layer. This will squash the range of each neuron in the layer to between -1 and 1. Such nonlinear elements provide a network with the ability to make soft decisions. The linear sigmoid (Equation 3) substitutes the intermediate portion of the sigmoid by a line with slope β, making it a piecewise linear approximation of the sigmoid. This component is more computationally efficient than the sigmoid axon (it is much easier to compute the map).

$$f(x_i,w_i) = \tanh[x_i^{lin}] \tag{2}$$

$$f(x_i,w_i) = \begin{cases} 0 & x_i^{lin} < 0 \\ 1 & x_i^{lin} > 1 \\ x_i^{lin} & \text{else} \end{cases} \tag{3}$$

Where $x_i^{lin} = \beta x_i$

There are several criteria such as the coefficient of determination (R^2), mean square error (MSE), normalized mean-squared error (NMSE) and mean absolute error (MAE), to evaluate the ANN model, which were calculated by using Equations 4 to 8 (Fathi *et al.*, 2009).

$$MSE = \frac{1}{N}\sum_{i=1}^{N}(O_i - T_i)^2 \tag{4}$$

$$NMSE = \frac{1}{\sigma^2}\frac{1}{N}\sum_{i=1}^{N}(O_i - T_i)^2 \tag{5}$$

$$MAE = \frac{1}{N}\sum_{i=1}^{n}|O_i - T_i| \tag{6}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{N}[O_i - T_i]^2}{\sum_{i=1}^{N}[O_i - T_m]^2} \tag{7}$$

Where O_i is the i^{th} real value, T_i is the i^{th} predicted value, N is the number of data, σ^2 is the variance, and

$$T_m = \frac{\sum_{i=1}^{N} O_i}{N} \tag{8}$$

Sensitivity analysis is a method for extracting the cause and effect relationship between the inputs and outputs of the network. The network learning is disabled during this operation such that the network weights are not affected.

2. Materials and methods

Red Cabbage used as raw material was purchased from the local market of Gorgan city in Golestan province of Iran. The essential oils, including cumin (*Cuminum cyminum)*, fennel (*Foeniculum vulgare*) and clove (*Syzygium aromaticum*), utilized in this study were obtained from the Ddepartment of Food Science and Technology of the Ferdousi University of Mashhad, Iran. The oils were added to distilled water as 0.2, 0.1, 0.075 and 0.05% (v/v) by vigorously shaking.

Crude vegetables extracts

Ten grams of each vegetable were cleaned and thoroughly washed. The vegetables were then chopped and homogenized for 3 min. The amount of water added during homogenation of vegetables was 30 ml. All steps were carried out at 4 °C. The slurry was filtered and solid particles was separated and centrifuged at 10,000×*g* for 15 min at 4 °C. The supernatant, which contained POD activity, was used as the enzyme source for the experiment.

Determination of enzyme activity

POD activity was determined spectrophotometrically at 25 °C with a UV 1601 PC UV-visible spectrometer (Shimadzu Corporation, Kyoto, Japan) at 470 nm using guaiacol as the substrate and H_2O_2 as the hydrogen donor (Hemeda and Klein, 1990). The substrate mixture contained 10 ml of 1% guaiacol, 10 ml of 0.3% hydrogen peroxide and 100 ml of 0.05 M sodium phosphate buffer (pH 6.5). The reaction cuvette contained 2.87 ml substrate mixture, 0.1 ml crude extract, and 0.03 ml antioxidant solution in a total volume of 3 ml. In order to use the right levels of enzymatic activity, the assay volume was adjusted to an adequate dilution to ensure linearity of the assay. Since POD activity assay using guaiacol as a substrate is very sensitive and rapid, it is important to use the right levels of enzymatic activity in the extract. For red cabbage the POD activity was measured at different dilution ratios. Finally, a dilution ratio of 25 ml/100 ml (v/v) was selected for red cabbages. A reagent blank was prepared with 0.03 ml deionized water instead of antioxidant (control sample). One unit of activity was defined as a change in absorbance of 0.001 min^{-1}.

Artificial neural network model development

In total, 240 data were collected for the three different essential oils (cumin, fennel and clove), four antioxidant concentrations (50, 75, 100 and 200 ml/100 ml) and 20 durations of enzyme activation (0, 20, 40… 400 seconds) as input neuron and enzyme activity as output neuron (Figure 1). First, the data order was randomized, then the data of essential oils were translated by binary translator and after that the data were divided into three partitions. The first partition (training data) was used to perform the training of the network (40% of the data). The second one (cross validation data) was used to evaluate the prediction quality of the network during the training (30% of the data). For the purpose of estimating the performance of the trained network on new data, a third partition, which never was seen by the ANN during the training and cross-validation process, was used for testing (30% of the data). During training, the momentum value was fixed at 0.7, and the learning rate was determined at level 1 on the hidden layer and 0.1 at the output layer. The training process was carried on for 65,000 epochs or until the cross-validation data's MSE, calculated by Equation 4, did not improve for 100 epochs to avoid over-fitting of the network (Fathi *et al.*, 2009). Since almost all of the problems in neural network modelling could be solved with one hidden layer

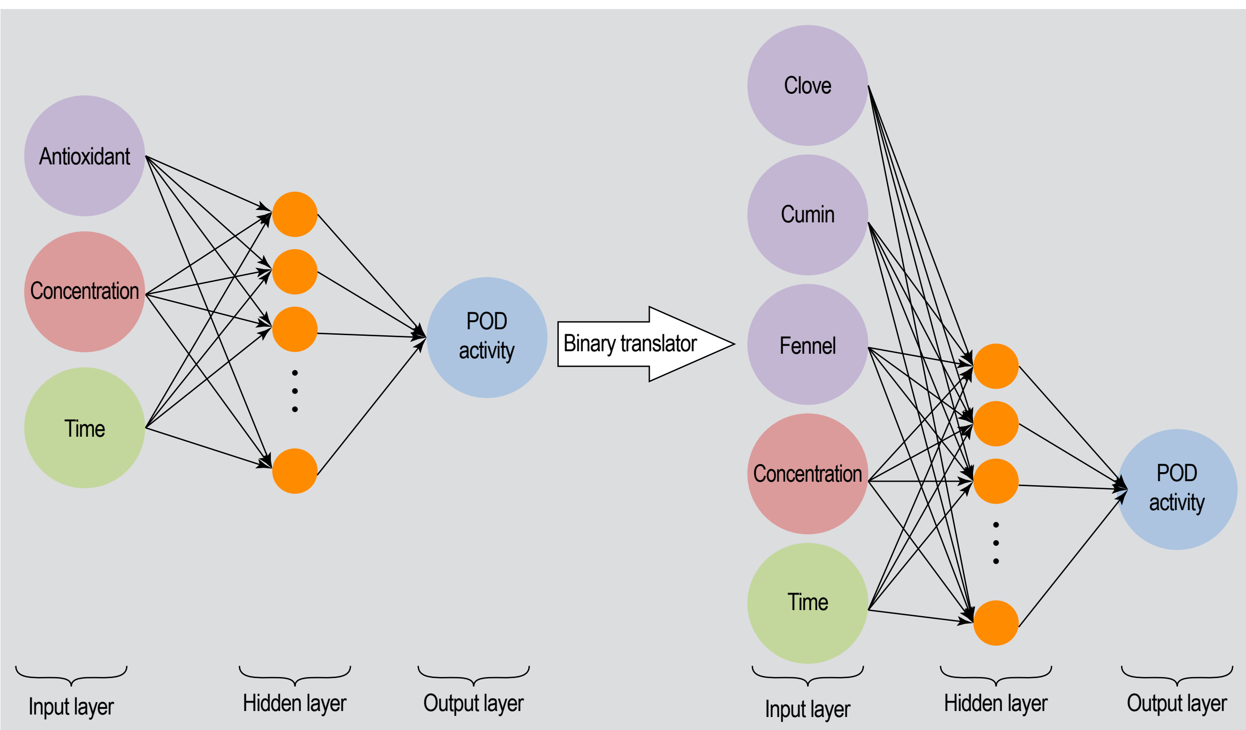

Figure 1. Structure of the feed-forward multi-layer artificial neural network for estimating peroxidase (POD) activity.

(Baş and Boyacı, 2007; Baş *et al.*, 2007a,b; Fathi *et al.*, 2009; Kashaninejad *et al.*, 2009; Razmi-Rad *et al.*, 2007), hence, in this study the ANN model was developed with one hidden layer. To determine the optimum network, the size of hidden layer was changed and simultaneously the resulting changes in the MSE were recorded. For testing of the final network and doing the sensitivity test about the mean, the best weights obtained during the training of the network were used. Evaluation of the performance of the trained network was based on the accuracy of the network in the test partition.

3. Result and discussion

The ANN parameters used for the prediction of the POD activity in red cabbage are shown in Table 1. The optimal number of hidden layers and number of neurons in the hidden layers were selected by using a trial and error method based on minimizing the difference between the estimated ANN outputs and the experimental values. The results are shown in Table 2.

It was found that ANN with one hidden layer and 21 hidden neurons had the minimum MSE for training and cross validation: 7.9815×10^{-6} and 2.9123×10^{-5}, respectively. The predicted POD activity versus the actual POD activity for the testing data set was plotted (Figure 2) and the equation of linear regression and R^2 were determined. The sensitivity of all parameters was determined and plotted (Figure 3). The POD activity was shown to have the highest sensitivity to time. It is clear that all chemical reactions are dependent on time. Of the three essential oils, cumin was shown to have the highest effect on the POD activity due to high antioxidant properties.

Table 1. The best structure and optimum values of the artificial neural network (ANN) produced in the testing stage.

ANN structure		3-21-1
ANN model		MLP
Step size	**Hidden layer**	1
	Output layer	0.1
Momentum	**Hidden layer**	0.7
	Output layer	0.7
Transfer function	**Hidden layer**	Tanh
	Output layer	Linear sigmoid
Epoch		65,000
Testing	**MSE**	0.0002629
	NMSE	0.0026579
	MAE	0.0102657
	R^2	0.9974

MLP = multilayer perceptron; MSE = mean square error; NMSE = normalized mean-squared error; R^2 = coefficient of determination.

Table 2. Minimum mean square error (MSE) for the prediction of the peroxidase activity with different numbers of neurons in the hidden layer during training and cross validation.

Number of neurons in hidden layer	Minimum MSE	
	Training	Cross validation
2	0.001242154	0.001393705
3	0.000263449	0.00034895
4	6.6609E-05	0.000111743
5	3.36074E-05	7.09705E-05
6	4.45928E-05	7.70141E-05
7	2.98847E-05	9.1206E-05
8	2.49714E-05	0.00011427
9	1.5971E-05	4.99593E-05
10	1.9747E-05	4.422E-05
11	2.2407E-05	4.60941E-05
12	2.15988E-05	4.94446E-05
13	1.58731E-05	5.3614E-05
14	2.55036E-05	4.19363E-05
15	1.07515E-05	4.22857E-05
16	9.8512E-06	5.85819E-05
17	1.56989E-05	3.18658E-05
18	1.36463E-05	4.88243E-05
19	1.70279E-05	4.89604E-05
20	1.18729E-05	6.5632E-05
21	7.9815E-06	2.9123E-05
22	1.05224E-05	3.31315E-05
23	2.37921E-05	3.86497E-05
24	1.494E-05	3.23449E-05
25	1.01024E-05	4.86083E-05

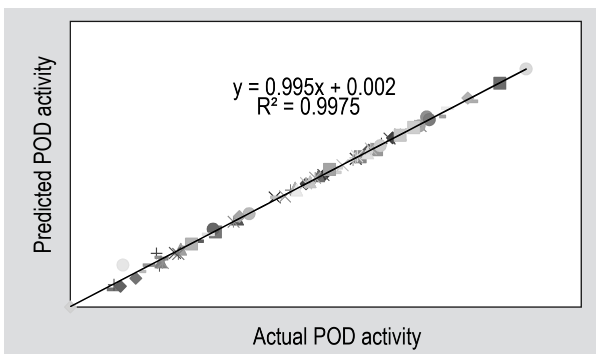

Figure 2. Predicted peroxidase (POD) activity versus actual POD activity for the testing data set.

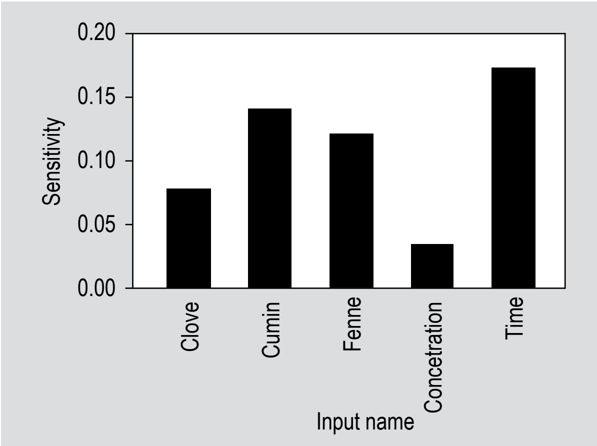

Figure 3. Sensitivity of peroxidase activity to every artificial neural network input.

4. Conclusion

The POD activity of vegetables and fruits is a linear phenomenon which depends on several factors. ANNs are mathematical models whose architecture has been inspired by biological neural networks. ANNs are very appropriate for the modelling of linear and non-linear processes. The advantage of ANNs over conventional methods, like regression analysis, is time and cost saving. Also, the ANNs can consider more input parameters and the performance is better than of conventional methods. The ANN model in this study predicted a POD activity with a MSE of 0.0002629 and a good correlation between predicted and experimental data (R^2=0.9974). These results show the ability of the ANN technology for predicting POD activity of red cabbage under natural antioxidants. The results show that POD activity had the highest sensitivity to time and of the three essential oils, cumin was shown to have the highest effect on POD activity due to high antioxidant properties.

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References

Aghajani, N., Kashaninejad, M., Dehghani, A.A. and Daraei Garmakhany, A., 2012. Comparison between artificial neural networks and mathematical models for moisture ratio estimation in two varieties of green malt. Quality Assurance and Safety of Crops & Foods 4: 93-101.

Aruoma, O.I., Spencer, J.P.E., Rossi, R., Aeschbach, R., Kahn, A., Mahmood, N., Munoz, A., Murcia, A., Butler, J. and Halliwell, B., 1996. An evaluation of the antioxidant and the antiviral action of extracts of rosemary and provencal herbs. Food and Chemical Toxicology 34: 449-456.

Baş, D. and Boyacı, I.H., 2007. Modeling and optimization II: comparison of estimation capabilities of response surface methodology with artificial neural networks in a biochemical reaction. Journal of Food Engineering 78: 846-854.

Baş, D., Dudak, F.C. and Boyacı, I.H., 2007a. Modeling and optimization III: reaction rate estimation using artificial neural network (ANN) without a kinetic model. Journal of Food Engineering 79: 622-628.

Baş, D., Dudak, F.C. and Boyacı, I.H., 2007b. Modeling and optimization IV: investigation of reaction kinetics and kinetic constants using a program in which artificial neural network (ANN) was integrated. Journal of Food Engineering 79: 1152-1158.

Bryjak, J., Ciesielski, K. and Zbicinski, I., 2004. Modelling of glucoamylase thermal inactivation in the presence of starch by artificial neural network. Journal of Biotechnology 114: 177-185.

Daraei Garmakhany, A., Mirzaei, H.O., Aghajani, N. and Kashiri, M., 2010. Investigation of natural essential oil antioxidant activity on peroxidase enzyme in selected vegetables. Journal of Agricultural Science and Technology 4: 78-82.

Fathi, M., Mohebbi, M. and Razavi, S.M., 2009. Application of image analysis and artificial neural network to predict mass transfer kinetics and color changes of osmotically dehydrated kiwifruit. Food and Bioprocess Technolology 4: 1357-1366.

Gunes, B. and Bayindirli, A., 1993. Peroxidase and lipoxygenase inactivation during blanching of green beans, green peas and carrots. LWT - Food Science and Technology 26: 406-410.

Hemeda, H.M. and Klein, B.P., 1990. Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. Journal of Food Science 55: 184-186.

Kashaninejad, M., Dehghani, A.A. and Kashiri, M., 2009. Modeling of wheat soaking using two artificial neural networks (MLP and RBF). Journal of Food Engineering 91: 602-607.

Kashiri, M., Daraei Garmakhany, A. and Deghani, A.A., 2012. Modeling of sorghum soaking using artificial neural networks (MLP). Quality Assurance and Safety of Crops & Foods 4: 179-184.

Lee, H.C. and Klein, B.P., 1989. Evaluation of combined effects of heat treatment and antioxidant on peroxidase activity of crude extract of green peas. Food Chemistry 32: 151-158.

Linko, S., Zhu, Y. and Linko, P., 1999. Applying neural networks as software sensors for enzyme engineering. Trends in Biotechnology 17: 155-162.

Razmi-Rad, E., Ghanbarzadeh, B., Mousavi, SM., Emam-Djomeh, Z. and Khazaei, I., 2007. Prediction of rheological properties of Iranian bread dough from chemical composition of wheat flour by using artificial neural networks. Journal of Food Engineering 81: 728-734.

Rudra Shalini, G., Shivhare, U.S. and Basu, S., 2008. Thermal inactivation kinetics of peroxidase in mint leaves. Journal of Food Engineering 85: 147-153.

Rui, M.S.C., Vieira, M.C. and Silva, C.L.M., 2006. Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*). Journal of Food Engineering 72: 8-15.

Yang, B.Y., Gray, J.S.S. and Montgomery, R., 1996. The glycans of horseradish peroxidase. Carbohydrate Research 287: 203-212.