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Using Neural Networks to Estimate the Losses of Ascorbic Acid, Total Phenols, Flavonoid, and Antioxidant Activity in Asparagus during Thermal Treatments

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Artificial neural networks (ANNs) with back-propagation algorithm were developed to predict the percentage loss of ascorbic acid, total phenols, flavonoid, and antioxidant activity in different segments of asparagus during water blanching at temperatures ranging from 65 to 95 °C as a function of blanching time and temperature. In this study, the one-hidden-layer ANNs are used, and the number of neurons in the hidden layer were chosen by trial and error. Optimized ANN models were developed for predicting nutrient losses in bud, upper, middle, and butt segments of asparagus. ANN models were then tested against an independent data set. Our results showed that the predicted values of the correlation coefficients between experimental and ANNs ranged from 0.8166 to 0.9868. Therefore, ANNs could be potential tools for the prediction of nutrient losses in vegetables during thermal treatments.

KEYWORDS: ***Asparagus officinalis*** **L.; ANN; ascorbic acid; total phenols; flavonoid; scavenging activity; thermal treatments**

INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is a green vegetable with high antioxidant activity among the commonly consumed vegetables (*1*). The antioxidants in asparagus include ascorbic acid, flavones, phenols, glutathione, etc. (*2*). Antioxidants can scavenge free radicals and protect the human body from oxidative stress, which is the main cause of some cancers and heart diseases (*3*). However, asparagus is an easily perishable vegetable characterized by a limited postharvest life, mainly because of its high respiratory activity, which continues after harvesting. Therefore, it is preserved by canning, pickling, freezing, and drying. Blanching, a thermal process preceding freezing or canning of vegetables, is necessary to inactivate shelf-life-limiting enzymes and to exhaust gas from the plant tissue (*4*). Hot water blanching is by far the most popular and commercially adopted process as it is the simplest and most economical technique. Blanching, however, has some adverse effects, such as pigment modifications, tissue softening, and nutrient losses. Many researchers have observed the dramatic blanching effect on the degradation of vegetables nutrient content and antioxidant properties (*5*−*8*). As far as we know, by taking into account these recent works, information regarding the mathematical model to predict the nutrient content and antioxidant properties of vegetables during thermal treatments is still scarce.

The artificial neural network (ANN) is a set of mathematical methods, often encompassed with artificial intelligence, which in some way attempts to mimic the functioning of the human brain (*9*). In the last two decades, ANN has revealed its huge potential in many areas of science and engineering (*10*−*13*) because of its exceptional function of self-organizing, self-study, fault tolerance, and high robustness. Recently, interest in using neural networks as a modeling tool in agriculture and food technology is also increasing. It has been successfully used in several food-processing technologies such as drying technologies (*14*, *15*), postharvest technology (*16*), food rheology (*17*), microbial predictions (*18*), fermentation (*19*), and thermal processing (*20*, *21*).

Therefore, the aim of this study was to evaluate the percentage loss of ascorbic acid, total phenols, flavonoid, and antioxidant activity of different segments of green asparagus during blanching in water through mathematical modeling based on the ANN. The results will help to define optimal blanching conditions for maximum quality retention, and the success of this research will provide the food industries with modeling and simulation for nutrient loss control in vegetables during thermal treatments.

MATERIALS AND METHODS

Sample Preparation and Blanching Process. Fresh asparagus (*Asparagus offcinalis* L. var. Grande) was harvested from a local farm in Jinhua (Zhejiang, P.R. China) and transported by refrigeration at 8 °C for 30 min to the laboratory. Spears of the same diameter (0.8−1.0 cm) at the base and length (20 cm) were used in our experiment. The spears, after being sorted for size and length, were washed with tap water and drained. Sixty asparagus spears were placed in distilled water baths set at 60 °C, 65 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, and 95 °C. Nine spears were removed at the time intervals listed in **Table 1** and immediately cooled in

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running water at 15 °C. After cooling, nine spears were randomly divided into three groups (three spears per group). Prior to ascorbic acid, total phenols, flavonoid, and scavenging capacity measurements, asparagus spears at room temperature were cut into four segments (bud, upper, middle, and butt segments) as shown in **Figure 1**. All experiments were repeated three times (one time per group), and the results were reported as average. In addition, the samples were kept at below 0 °C until analysis was done to prevent nutrient losses.

Chemicals and Reagent. 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) free radical (purity ≥90%) and rutin (purity ≥95%) were of analytical grade and purchased from Sigma-Aldrich (St.Louis, MO, USA). Gallic acid (purity ≥98%), ascorbic acid (purity ≥99%), Folin−Ciocalteu reagent (purity ≥99%), and 2,6-dichlorophenol indophenol (purity ≥99%) were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China).

Ascorbic Acid (AA) Determination. The AA content in different segments of asparagus was determined upon the basis of quantitative discoloration of the 2,6-dichlorophenol indophenol titrimetric method as described in AOAC (*22*). Results of AA content were expressed as milligram ascorbic acid per 100 mL juice. The AA content was measured in triplicate.

Total Flavonoid (TF) Content Determination. The TF content was evaluated by a colorimetric assay according to the method of Bonvehí et al. (*23*), with modifications. Briefly, 0.5 mL solution of each plant extract in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 2% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer. A standard curve ($y = 0.4564x$; $R^2 = 0.9942$; SD = 0.0046) was prepared with rutin. The TF content was measured in triplicate and expressed as rutin equivalents in mg g^{-1} dry weight of asparagus.

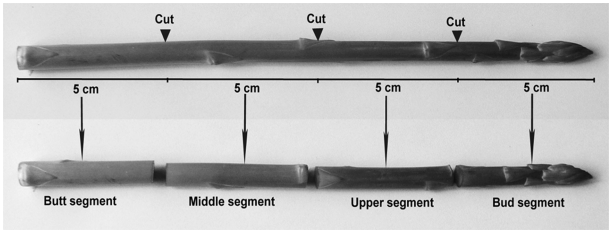

Figure 1. Specimens for measuring ascorbic acid, total phenols, flavonoid, and DPPH radical scavenging activity.

Table 1. Blanching Time and Temperature for Fresh Asparagus

temperature (°C)	blanching time (min)			
60	40	80	120	160
65	35	70	105	140
70	30	60	90	120
75	25	50	75	100
80	20	40	60	80
85	15	30	45	60
90	10	20	30	40
95	5	10	15	20

Total Phenolic (TP) Content Determination. The TP content was determined colorimetrically using Folin−Ciocalteau reagent, as described by Emmons, Peterson, and Paul (*24*), with modifications. Total phenolic assay was conducted by mixing 8.3 mL of deionized water, 0.5 mL of extracts, 0.7 mL of 20% Na_2CO_3, and 0.5 mL of Folin−Ciocalteu reagent. After 40 min of reaction in a water bath at 40 °C, the absorbance at 755 nm was measured using a spectrophotometer. A standard curve ($y = 0.0903x - 0.0011$; $R^2 = 0.9969$; SD = 0.0015) was prepared with gallic acid. Final results were expressed as gallic acid equivalents (GAE) mg/g of dry asparagus. The TP content was measured in triplicate.

DPPH Radical Scavenging Activity (SA) Determination. The free radical scavenging activity of plant extracts was evaluated using the stable radical DPPH as described by Maisuthisakul et al. (*25*). DPPH radical in methanol (5 mM) was prepared, and this solution (100 μL) was added to sample solutions in methanol (4.9 mL). After 30 min in the dark, absorbance was measured at 517 nm. SA was measured in triplicate samples. The percentage of DPPH radical scavenging activity of each plant extract was calculated using the following equation:

$$\text{Scavenging activity } (\%) = \left[\frac{A_0 - (A_1 - A_s)}{A_0}\right] \times 100 \qquad (1)$$

where A_0 is the absorbance of the control solution (containing only DPPH); A_1 is the absorbance of the DPPH solution containing plant extract; and A_s is the absorbance of the sample extract solution without DPPH.

ANN Analysis. The ANN is a mathematical algorithm inspired by studies of the brain and nervous systems in biological organisms, which has the capability of relating the input and output parameters, learning from examples through iteration, without requiring prior knowledge of the relationships between the process variables (*26*). A feed forward network structure with input, output, and hidden layers was used in this study as shown in **Figure 2**. The input layer consisted of two neurons which corresponded to blanching time and temperature. The output layer had one neuron representing the percentage loss of nutrient (eg, ascorbic acid, total phenols, flavonoid, or antioxidant activity). The neural network can have more than one hidden layer; however, theoretical works have shown that a single hidden layer is sufficient for the neural network to approximate any complex nonlinear function (*27*, *28*). Hence, the one-hidden-layer ANNs are used in this study. The number of neurons within each of these layers varied from 2 to 10. The back-propagation algorithm was utilized in the training of ANN models (*27*). A hyperbolic tangent sigmoid was used as the transfer function in the hidden layer and output layer.

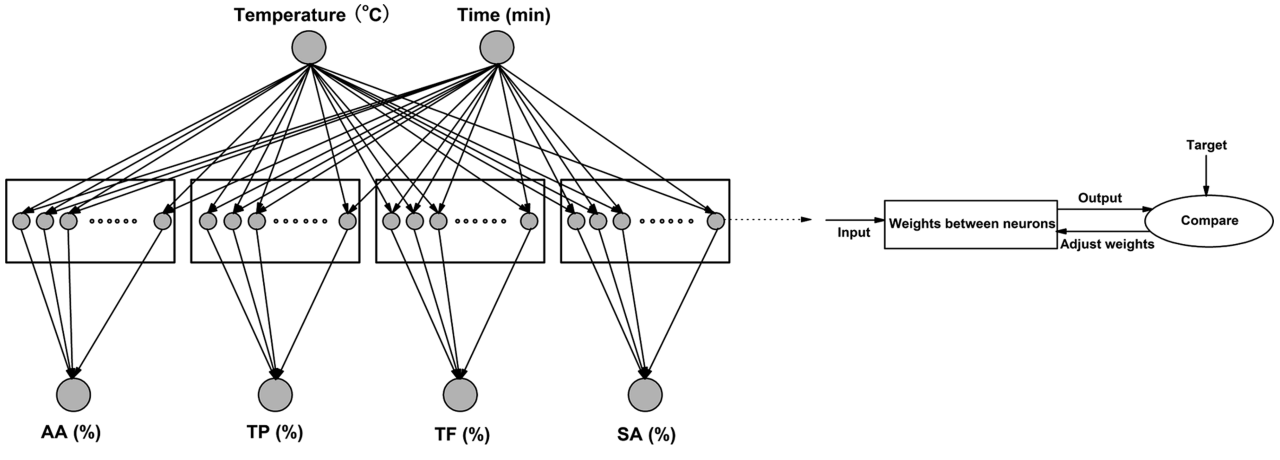

Figure 2. Schematic diagram of artificial neural networks for the prediction of the percentage loss of ascorbic acid, total phenols, flavonoid, and DPPH radical scavenging activity used in this study.

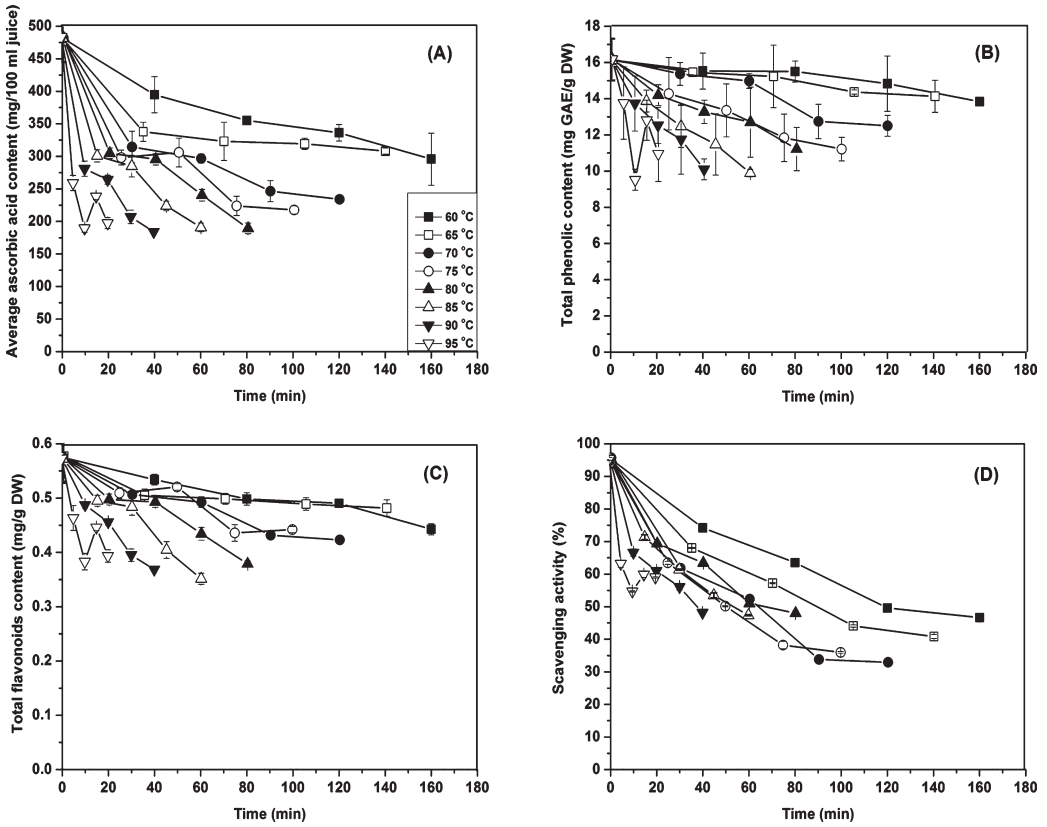

Figure 3. Water blanching effect on ascorbic acid (**A**), total phenols (**B**), flavonoid (**C**), and DPPH radical scavenging activity (**D**) of the bud segment of asparagus.

Minimization of error was accomplished using the Levenberg−Marquardt (LM) algorithm (*29*, *30*). The numerical values of the input and output variables used by the ANN were normalized values in the range 0−1.

Training was based on a supervised method with back-propagation strategy and was finished when the mean square error (MSE) converged and was less than 0.1. If the MSE did not go below 0.1, training was completed after 1,000 epochs, where an epoch represents one complete sweep through all of the data in the training set. The ANN modeling program was designed and programmed under Matlab software, version 6.1.

The prediction performances of the various ANN models were compared using mean squared error (MSE). The MSE was calculated using the following equation:

$$MSE = \frac{1}{N}\sum_{i=1}^{N}(k_E - k_P)^2 \quad (2)$$

where N is the total number of data; k_P represents the predicted output from the neural network model for a given input, while k_E is the experimental value.

RESULTS AND DISCUSSION

Blanching is a heat treatment preferably in a wet medium either by steam or hot water which provides uniform heating and high heat transfer rate (*4*). Water blanching is by far the most popular and commercially adopted process because it is the simplest and most economical method. In addition, the household practice of cooking vegetables is usually in the presence of water. However, water blanching may have some negative effect on vegetable quality, such as excessive loss of texture, unwanted changes in color, and nutritional losses (*8*, *31*−*33*). In our study, the AA, TP, TF, and SA in green asparagus markedly decreased, depending on the blanching time and temperature. A representative graph for nutritional losses in the bud segment of asparagus is given in **Figure 3**, and the percentage losses of AA, TP, TF, and SA were calculated in the bud segment of asparagus and given in **Tables 2**−**4**. In recent years, many authors have developed kinetic models to describe the quality changes in vegetables during blanching (*34*−*38*). However, to our knowledge no previous study has been reported on using ANN to predict the percentage

Table 2. Learning Data Set[a] Used in the Development of ANNs

		percentage loss (%)			
temperature (°C)	time (min)	AA	TP	TF	SA
60	80	26.32	3.93	13.34	33.41
60	160	38.66	14.25	23.02	51.08
65	35	29.48	4.13	12.52	28.30
65	105	33.68	10.88	15.30	53.41
65	70	32.88	5.52	13.64	39.66
70	30	35.02	5.15	11.97	35.55
70	60	38.72	7.56	14.45	45.50
70	120	51.73	22.91	26.51	65.83
75	25	38.06	11.82	11.45	33.10
75	75	53.47	26.95	24.20	59.57
80	20	36.79	12.43	13.50	27.24
80	40	38.68	18.13	14.27	33.48
80	80	60.74	30.89	34.13	49.66
85	15	37.58	13.96	13.46	25.20
85	45	53.55	28.82	29.21	43.93
85	60	60.42	38.63	38.46	50.33
90	10	41.63	14.90	14.91	29.81
90	30	56.98	27.21	30.95	40.88
90	40	61.72	37.45	35.58	49.17
95	5	46.62	15.02	19.08	33.74
95	15	50.90	20.90	22.11	37.20
95	20	59.40	32.37	31.24	38.14

[a] These data were collected from bud segments of asparagus as representatives.

loss of ascorbic acid, total phenols, flavonoid, and antioxidant activity of different segments of green asparagus during blanching in water. So far, the application of neural networks in the food science area has broad coverage: sensory analysis, classifications, microbial predictions, thermal control, etc. (*17*, *18*, *21*, *39*, *40*). The purpose of this study was to prove that the ANN is a convenient and potential tool for the prediction of the quality changes of different segments of green asparagus during water blanching as a function of blanching time and temperature. The development of the ANN model involves three basic steps: training, validation, and prediction phases.

Table 3. Validating Data Set[a] Used in the Development of ANNs

		percentage loss (%)			
temperature (°C)	time (min)	AA	TP	TF	SA
60	40	18.10	3.78	7.14	22.18
65	140	36.01	12.35	16.61	56.84
75	100	54.78	30.79	23.12	61.93
80	60	50.12	21.65	24.47	46.52
90	20	45.10	22.41	20.36	35.68

[a] These data were collected from bud segments of asparagus as representatives.

Table 4. Testing Data Set[a] Used in the Development of ANNs

		percentage loss (%)			
temperature (°C)	time (min)	AA	TP	TF	SA
60	120	30.25	8.11	14.72	47.98
70	90	49.12	21.41	25.04	64.90
75	50	36.42	17.56	9.47	47.06
85	30	40.89	22.63	15.44	35.62
95	10	61.01	41.29	33.06	42.65

[a] These data were collected from bud segments of asparagus as representatives.

Training of the Neural Network. In our study, 75% of the data were used to generate the model, and validation was performed with 12.5% of the total data. In addition, the remaining 12.5% of the data were used in the prediction phase. **Table 2** shows the training set, which was presented to the network, and a back-propagation algorithm automatically adjusted the weights until the output response to input vector was as close as possible to the desired response (*39*). The one-hidden-layer ANNs are used in this study because many researchers have shown that a single hidden layer is sufficient to approximate any complex nonlinear function (*27*, *28*). The optimal number of nodes in the hidden layer was selected by using a trial and error method. **Figure 4** shows the changes of MSE in the prediction of the percentage loss of AA, TP, TF, and SA in the bud, upper, middle, and butt segments of asparagus with different numbers of neurons in the hidden layer. Our results showed that the optimal number of nodes in the hidden layer was 5, 5, 7, and 5 in the bud segment of asparagus, 5, 7, 5, and 7 in the upper segment, 8, 4, 6, and 7 in the middle segment, and 5, 5, 5, and 7 in the butt segment, for predicting the percentage loss of AA, TP, TF, and SA, respectively. The above models were developed for asparagus diameters varying from 0.8 to 1.0 cm and temperatures from 65 to 95 °C. These ANN models, however, did not consider temperatures below 65 °C and above 95 °C or diameters of asparagus below 0.8 cm and above 1.0 cm.

Validation and Prediction Phases. In validation and prediction phases, we used the selected topology of ANN models with

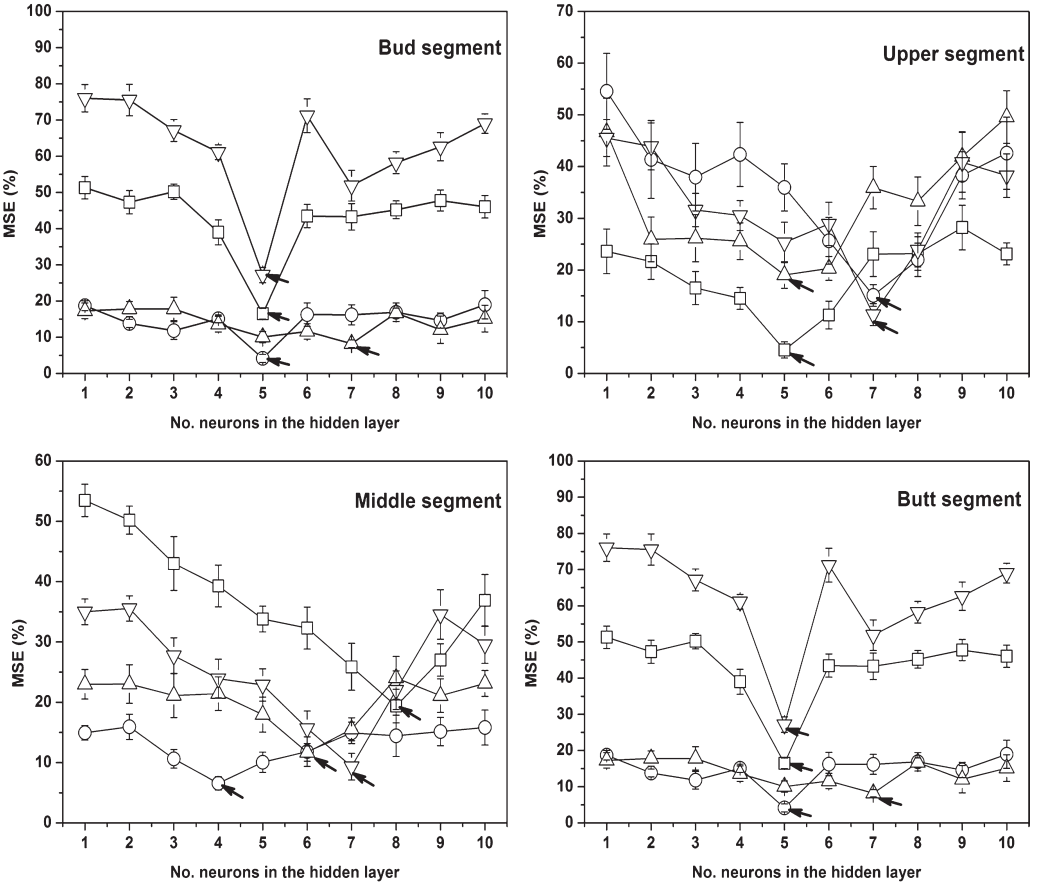

Figure 4. Mean squared error (MSE) in the prediction of the percentage loss of ascorbic acid (□), total phenols (○), flavonoid (▽), and DPPH radical scavenging activity (△) with different numbers of neurons in the hidden layer for bud, upper, middle, and butt segments of asparagus during blanching in water. The arrows signify the optimal number of nodes in the hidden layer.

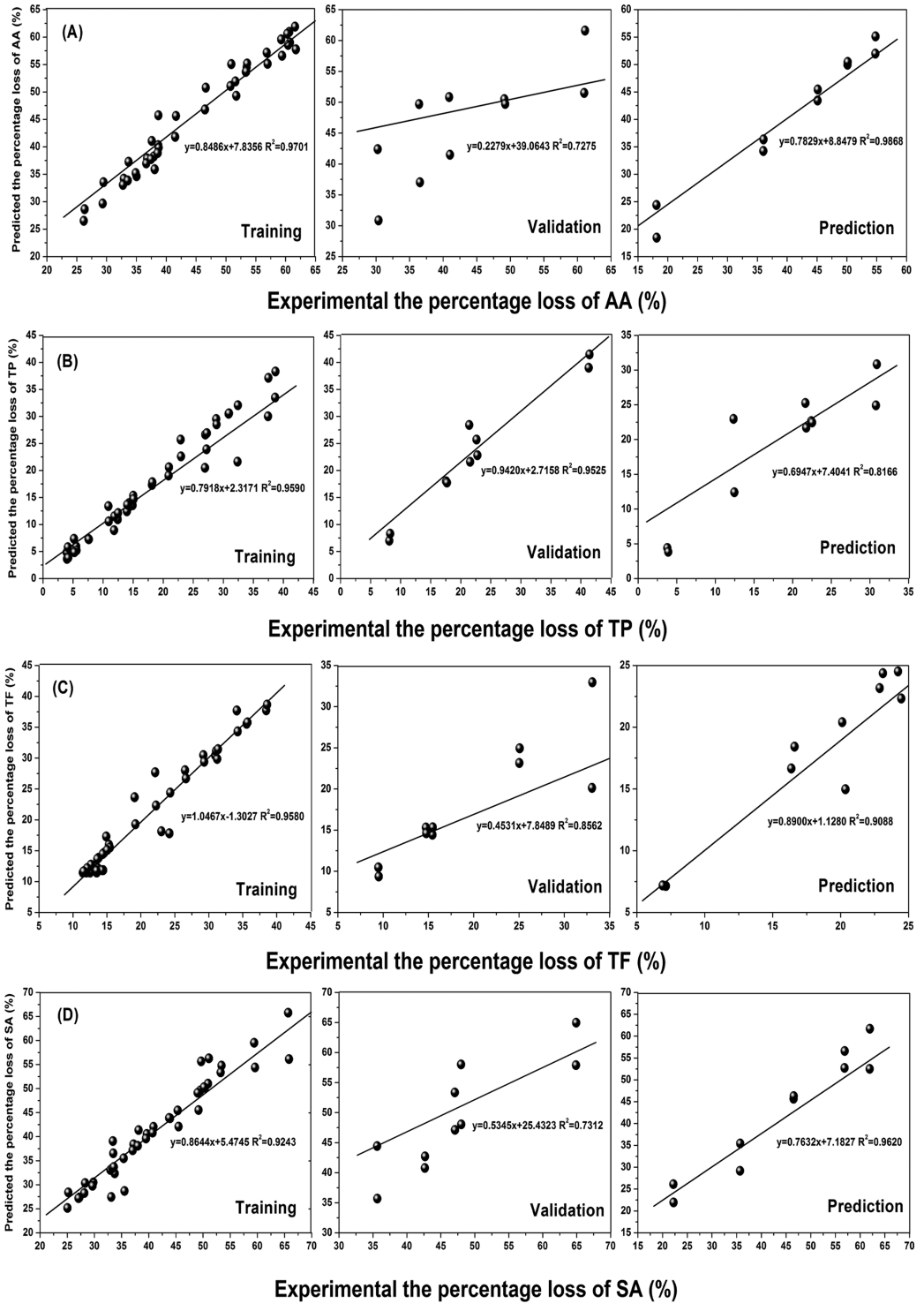

Figure 5. Correlation of experimental and predicted percentage loss of ascorbic acid (**A**), total phenols (**B**), flavonoid (**C**), and DPPH radical scavenging activity (**D**) with training and validation data sets, as well as the prediction data set for the bud segment of asparagus during water blanching using the optimal ANN.

Table 5. Mean Squared Errors (MSE) and the Correlation Coefficients (R^2) in the Prediction of the Percentage Loss of AA, TP, TF, and SA in Different (Bud, Upper, Middle, and Butt) Segments of Asparagus with the Optimal ANNs

	AA		TP		TF		SA	
	MSE (%)	R^2	MSE (%)	R^2	MSE (%)	R^2	MSE (%)	R^2
bud segment	10.6745	0.9868	51.2112	0.8166	7.6963	0.9088	32.8099	0.9620
upper segment	62.1037	0.8318	30.7957	0.8767	12.7976	0.8234	33.6940	0.8716
middle segment	28.2051	0.9205	44.4763	0.8568	4.6623	0.9780	75.3507	0.8665
butt segment	24.1384	0.9654	14.8777	0.8597	7.3191	0.9862	26.9330	0.9129

the previously adjusted weights. Representative graphs of the correlations between the predicted and experimental values in respect to the training set, validation set, and prediction set are shown in **Figure 5**, together with the full description of equations of the linear regression model. In addition, **Table 5** shows the MSE and the correlation coefficients (R^2) between experimental and ANN predicted values in the prediction of the percentage loss of AA, TP, TF, and SA in different (bud, upper, middle, and butt) segments of asparagus with the optimal ANNs. The optimal ANN could predict the percentage loss of AA, TP, TF, and SA in asparagus with the MSE of 10.6745%, 51.2112%, 7.6963%, and 32.8099% for the bud segment, 62.1037%, 30.7957%, 12.7976%, and 33.6940% for the upper segment, 28.2051%, 44.4763%, 4.6623%, and 75.3507% for the middle segment, and 24.1384%, 14.8777%, 7.3191%, and 26.9330% for the butt segment, respectively. In addition, the correlation coefficients between experimental and ANN predicted values ranged from 0.8166 to 0.9868, as shown in **Table 5**. Therefore, the ANN model as a potential tool can be used to determine the percentage loss of AA, TP, TF, and SA during water blanching.

In conclusion, the ANN, a convenient and potential tool, can be a promising method for modeling the nutrient losses of vegetables during water blanching. Therefore, the ANN seems to find application in the quality analysis of functional properties of food of plant origin for the prediction of not only the ascorbic acid, total phenols, flavonoid, and DPPH radical scavenging activity but also for the control of food quality during processing.

ABBREVIATIONS USED

AA, ascorbic acid; TP, total phenols; TF, total flavonoid; SA, DPPH radical scavenging activity.

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