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Artificial Neural Network for Production of Antioxidant Peptides Derived from Bighead Carp Muscles with Alcalase

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Summary

Controlled enzymatic modification proteins are currently being used as good sources of bioactive protein ingredients, and hydrolysates derived from bighead carp muscles may serve as antioxidants through the control of the processing-related parameters. The antioxidant ability was evaluated with regard to the scavenging effect on free radical $DPPH^{\bullet}$, $OH^{\bullet}$ and $O_2^{\bullet-}$. Due to the robustness, fault tolerance, high computational speed and self-learning ability, artificial neural network (ANN) can be employed to build a predictive model for hydrolysis and optimize the hydrolysis variables: pH, temperature, hydrolysis time, muscle/water ratio and enzyme/substrate ratio (E/S) for the production of antioxidant peptides. Optimum conditions to achieve the maximum antioxidant ability were obtained. The hydrolysates, which scavenged most effectively the $DPPH^{\bullet}$, $OH^{\bullet}$ and $O_2^{\bullet-}$, were hydrolyzed for 4.8 h with an activity of alcalase of 4.8 AU/kg, for 6 h with 3.84 AU/kg and for 4.3 h with 4.8 AU/kg, at pH=7.5 and 60 °C. Their respective muscle/water ratio was 1:1.9, 1:1.4 and 1:1. The present study confirmed that ANN could be used to simulate the hydrolysis process and predict hydrolysis conditions under which the hydrolysates could show the most effective scavenging ability on $DPPH^{\bullet}$, $OH^{\bullet}$ and $O_2^{\bullet-}$.

Key words: antioxidant peptides, artificial neural network (ANN), bighead carp, enzymatic hydrolysis

Introduction

Protein hydrolysate may be a source of bioactive peptides, which are short chain peptides with certain biological properties such as angiotensin converting enzyme (ACE) inhibition, antioxidant ability and antithrombosis. Within the sequence of the parent protein, the peptides are inactive. However, after enzymatic hydrolysis, the bioactivity can be released (*1*). Antioxidant activities have been found in hydrolyzed proteins from many sources such as soybean, milk casein, oil seed protein and yellowfin sole frame protein (*2,3*). Unfortunately, very little information about antioxidant peptide from fish by-products is available (*4*). Morato *et al.* (*5*) reported that the original protein, the type of hydrolysis and peptide chain length affected the bioactivity of peptides. Fish protein was chosen for this study as the original protein due to its high nutritional value, great susceptibility to the catalytic action and market availability (*6*). Currently, hake, shark, sardine, herring, crayfish, capeline, dogfish and Atlantic salmon have been studied for making fish protein hydrolysates (FPH) (*7,8*). The bighead carp (*Aristichthys nobilis*), one of the biggest cultivated freshwater species in China, attracts considerable interest. Many people consume the bighead carps for the purpose of good nutrition and delicious flavour of

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fish heads. However, other parts of the bighead carps are sold at very low price, even though they contain high-quality proteins. To enhance the usage of the bighead carps, enzymatic digestion of muscle proteins can be used to produce FPH as ideal sources of bioactive peptides. Because the bioactive peptides are continuously formed and degraded during hydrolysis while the bioactivity is attributed to the combination of these peptides, controlling the process is very important (*9*). The compositions of the peptides resulting from hydrolysates depended on the specificity of the enzyme used and on the processing conditions such as pH and temperature (*10*). To optimize the hydrolysis for maximum bioactivity, a model involving the effect of different variables should be built. However, it was difficult to build the model because of the complicate relationship between the bioactive properties and the hydrolysis conditions (*9*).

Artificial neural network (ANN), which has been recognized as a powerful tool capable of performing better than conventional mathematical models, particularly for the case of nonlinear and multiple processing systems, is one of the widely studied areas within artificial intelligence (*11*). ANN is inspired by biological model and the building blocks are neurons that are combined into layers. The input is received and weighed according to weighing factors, and the resulting quantities are summed up (*12*). Without prior detailed knowledge of the relationship of processing variables, ANN could also relate the input to output parameters by learning from provided example and adapting itself through the learning stage. Due to the robustness, fault tolerance, high computational speed and self-learning ability, well-trained ANN can be employed in food industry, such as baking (*13*), drying (*14*), thermal processing (*15*), ultrafiltration (*16*) and fermentation (*17,18*). Compared to empirical models, ANN is considered to be more advantageous because it is robust to noise and can accommodate multiple-input and multiple-output systems. In most cases, it has been demonstrated that ANN models can perform better than the conventional ones based on regression, statistical or parametric models (*19,20*). Many kinds of networks are developed with different properties and application. Among these, back-propagation (BP) multilayer neural network is the most common and convenient tool.

The aim of this study was to employ the ANN to build the hydrolysis model of bighead carp muscles with alcalase, and then to optimize production conditions for the maximum antioxidant peptides. Some properties of the hydrolysates were also investigated.

Materials and Methods

Reagents

Alcalase 2.4 L (an activity of 2.4 AU/mL according to Novo Enzyme Co., China) was used because it is commercial, inexpensive and nonspecific with endopeptidase activity obtained from *Bacillus licheniformis*. 1,1-Diphenyl-2-picrylhydrazyl (DPPH$^\bullet$) free radicals and 2-deoxy--D-ribose were purchased from Sigma Chem. Co. Other chemicals were of analytical grade.

Bighead carp hydrolysates

Comminuted and defatted bighead carp muscles were used as substrates according to Quaglia and Orban (*21*), with minor modifications. The hydrolysis was performed under different conditions with respect to pH, temperature, hydrolysis time, enzyme/substrate ratio (E/S) and muscle/ water ratio (mass per volume), summarized in Table 1. The bighead carps without heads (provided kindly by Zhongshan Foodstuff and Aquatic Product Import Export Group Co., China) were eviscerated and washed with water, comminuted in a meat grinder and defatted by extracting for 30 min with isopropanol (1:1, volume per mass) 3 times at 46 °C. The protein content of the substrate after being defatted was 18.65 %. The residue was homogenized in distilled water with a blender, then the pH was adjusted, according to the design, with ammonia (pH=6.5–8), and finally subjected to enzymatic hydrolysis (according to the required E/S). After hydrolysis at different temperatures by rotating at 100 rpm, the reaction was terminated by inactivating the enzyme for 20 min at 100 °C in a water bath. The crude hydrolysate was then centrifuged at 10 000 rpm for 20 min to remove insoluble fragments, while the soluble phase was decanted, freeze-dried and kept at –20 °C for further use.

Methods

The antioxidant abilities of hydrolysates were evaluated as the scavenging activities with 1,1-diphenyl-2-picrylhydrazyl free radicals (DPPH$^\bullet$), hydroxyl radicals (OH$^\bullet$) and superoxide anion radicals ($O_2^{\bullet-}$). Food grade antioxidants *t*-butyl-hydroquinone (TBHQ) and ascorbic acid (Vc), used as references, were commented. All determinations were performed in triplicate and the results were the average of triplicate trials.

Scavenging ability on DPPH$^\bullet$ radicals

The scavenging effect of hydrolysates on DPPH$^\bullet$ was measured according to Wu *et al.* (*22*), with some modifications. A volume of 1.5 mL of each sample (1.3 mL of distilled water and 0.2 mL of hydrolysate) was added to 1.5 mL of 0.1 mmol/L DPPH$^\bullet$ in 95 % ethanol. The mixture was shaken and left for 30 min at 25 °C, and the absorbance of the resulting solution was measured at 517 nm. A lower absorbance represented a higher DPPH$^\bullet$ scavenging activity. The mixture of 1.5 mL of DPPH$^\bullet$ and 1.5 mL of water was used as control. The scavenging ability on DPPH$^\bullet$ was expressed as an inhibition/%= =100·(control absorbance–sample absorbance)/control absorbance.

Scavenging ability on OH$^\bullet$ radicals

The scavenging effect of hydrolysates on OH$^\bullet$ was measured by the deoxyribose method (*23*) with modifications. OH$^\bullet$ radicals were generated from Fenton reaction. A volume of 0.2 mL of 10 mmol/L $FeSO_4$ and 0.2 mL of hydrolysate were added to a 30-mL test tube and mixed for 5 min. A volume of 1.0 mL of 10 mmol/L 2-deoxy-D-ribose was then added, with a total volume of 1.6 mL using phosphate buffer (PBS, pH=7.4). Finally, 0.4 mL of H_2O_2 as promoter were added. After the mixture was incubated for 1 h at 37 °C, 1 mL of 0.8 % thio-

Table 1. The input and output of learning samples of ANN

	Input					Output/%		
	x_1	x_2/°C	x_3*	x_4/h	x_5**	DPPH$^\bullet$	OH$^\bullet$	$O_2^{\bullet-}$
1	1 (pH=6.5)	1 (50)	1 (1.2 AU/kg)	1 (3)	1 (1:1)	60.61	60.455	48.27
2	1	2 (55)	2 (2.4 AU/kg)	2 (4)	2 (1:2)	87.76	68.22	37.50
3	1	3 (60)	3 (3.6 AU/kg)	3 (5)	3 (1:3)	55.71	60.81	28.02
4	1	4 (65)	4 (4.8 AU/kg)	4 (6)	4 (1:4)	53.47	57.16	21.55
5	2 (pH=7.0)	1	2	3	4	72.24	64.42	26.30
6	2	2	1	4	3	88.78	65.84	26.73
7	2	3	4	1	2	76.12	67.87	38.37
8	2	4	3	2	1	73.27	65.28	50.43
9	3 (pH=7.5)	1	3	4	2	87.14	73.45	37.93
10	3	2	4	3	1	79.39	72.65	56.61
11	3	3	1	2	4	40.41	59.75	23.69
12	3	4	2	1	3	48.16	61.68	25.00
13	4 (pH=8.0)	1	4	2	3	85.92	58.58	33.19
14	4	2	3	1	4	42.45	56.81	22.85
15	4	3	2	4	1	60.61	77.06	38.37
16	4	4	1	3	2	85.10	66.10	32.33
17	3	3	4	1	1	68.72	71.38	53.78
18	3	3	4	2	1	76.19	73.56	57.32
19	3	3	4	3	1	77.85	72.98	56.24
20	3	3	4	4	1	72.39	72.84	51.09
21	3	3	4	1	2	78.96	67.98	40.41
22	3	3	4	2	2	87.89	68.05	42.04
23	3	3	4	3	2	88.83	69.20	41.22
24	3	3	4	4	2	87.65	73.89	38.50
γ/(mg/mL)								
Vc 2						93.10	–	95.18
Vc 0.2						90.38	–	89.28
TBHQ 0.04						71.06	45.28	27.72

*by volume per mass, **by mass per volume

barbituric acid (TBA) and 1 mL of 2.8 % trichloroacetic acid (TCA) were added. The mixture was heated for 15 min at 100 °C, then centrifuged for 15 min at 4 000 rpm. The absorbance of the red pigment in the supernatant was measured at 532 nm. The mixture without the hydrolysate was used as the control. The scavenging OH$^\bullet$ ability was expressed as an inhibition/%=100·(control absorbance–sample absorbance)/control absorbance.

Scavenging ability on $O_2^{\bullet-}$ radicals

The scavenging effect of hydrolysates on $O_2^{\bullet-}$ was measured by the pyrogallol autoxidation method (*24*) with modifications. A volume of 2.8 mL of 1 mmol/L Tris-HCl (pH=8.2) and 0.1 mL of hydrolysate were added to a test tube and mixed at 25 °C. A volume of 0.1 mL of pyrogallol solution (0.1 mmol/L) was then added, and the absorbance change rate of the mixture at 425 nm was recorded every 30 s. The mixture without hydrolysate was used as control. In this experiment, a stable autoxidation rate of control occurred within 3 min and the change rate of control absorbance at 425 nm was controlled at 0.05-0.06/min at 25 °C. The scavenging ability on $O_2^{\bullet-}$ was expressed as an inhibition/%=100·(control change rate–sample change rate)/control change rate.

Determination of degree of hydrolysis (DH) and peptide chain length (PCL)

α-Amino acid (A_N) was assayed by the formal titration procedure, while total nitrogen (T_N) was measured by the Kjeldahl method (*25*). The DH was calculated as $\mathrm{DH}/\% = \frac{A_{Nh} - A_{Nc}}{T_N \times P_f} \times 100$, where A_{Nh} and A_{Nc} were the percentage of amino nitrogen of the hydrolysate and intact bighead carp muscle, T_N referred to the mean percentage of total nitrogen of intact bighead carp muscle, while P_f was a correction factor for side chain nitrogen, which cannot be converted into amino nitrogen by hydrolysis of peptide bonds (*26*). According to Adler-Nissen (*27*), with the increase of DH, the peptide chain length could be expressed as PCL=1/DH.

Determination of nitrogen recovery (NR)

After the hydrolysis, the volume of the soluble fraction was recorded and nitrogen contents in these supernatants were determined by using the Kjeldahl method.

NR was calculated using the following equation NR/%= =100·(total nitrogen in the supernatant/total nitrogen in substrate) (*28*).

Determination of amino acid compositions

Amino acid concentration was analyzed using a Waters HPLC with PICO and TAG amino acid column. The sample was hydrolyzed for 22 h at 110 °C in 6 mol/L HCl under vacuum and the hydrolysate was then analyzed. Analysis of tryptophan was performed by hydrolysis of the sample in 3 mol/L mercaptoethane sulphonic acid at 110 °C under vacuum.

Calculation of protein efficiency ratio values (PER)

PER from the hydrolysates of bighead carp was calculated according to the equation (PER = –1.816 + 0.435 [Met] + 0.780[Leu] + 0.211[His] - 0.944[Tyr]), developed by Lee *et al.* (*29*) and Alsmeyer *et al.* (*30*).

Experimental design and ANN analysis

ANN was conducted using Matlab language. The type of ANN used was BP network with a 3-layer structure. It consisted of 5 artificial neurons in the input layer, 13 neurons in the hidden layer and 1 neuron in the output layer (Fig. 1). The input neurons x_1–x_5 stand for pH, temperature, E/S (volume per mass), hydrolysis time and muscle/water ratio (mass per volume) respectively, while the output neuron y stands for scavenging ability on different radicals, *i.e.* to evaluate the scavenging ability on free radicals DPPH•, OH• and $O_2^{\bullet-}$, three ANNs with the same structure were employed.

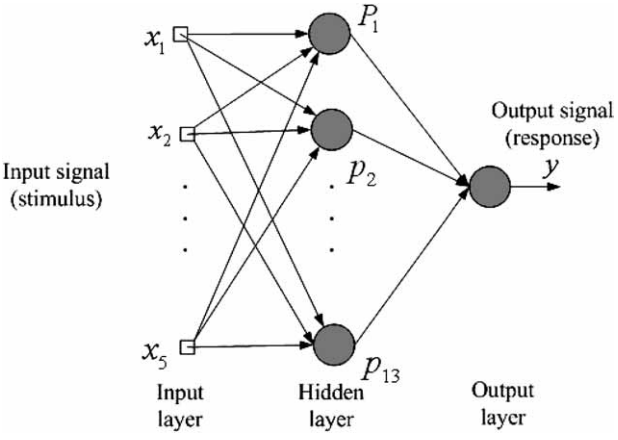

Fig. 1. The architecture of artificial neural network used for bighead carp protein hydrolysis

A supervised method of learning with BP strategy was used. The data were randomly divided into three sets, 24 objects for learning (Table 1), 4 objects for validating (Table 2) and finally 1 object for testing the maximum antioxidant ability. The learning of the ANN was executed with learning coefficient equal to 0.03 and mean-squared error equal to 10^{-3}. The sigmoid function and linear function were chosen for the activation functions of hidden and outer layers. When the predicted and desired values reached the goal, the learning process stopped. In the case of this network, learning was required to be completed in 10 000 epochs by BP methods. When the trained ANN model needed to be tested, the performance of the network was measured by R^2 and E_r, *i.e.*

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - y_{di})^2}{\sum_{i=1}^{n}(y_{di} - y_m)^2} \quad \text{and} \quad E_r = \frac{\sum|(y_i - y_{di})|}{n(y_{max} - y_{min})} \times 100$$

(*31*), where R^2 and E_r were the square of correlation coefficient and mean relative error, respectively; y_i was the predicted value by ANN model; y_{di} was the actual value, while n was the number of data and y_m was the average of the actual value; and y_{max} and y_{min} were the respective maximum and minimum values of all actual data. If the trained ANN performed well, the ANN could be used to predict the optimum condition for production of antioxidant peptides. The accuracy of the prediction can be tested by the actual experiments.

Results and Discussion

Building and training of BP network

The use of different methods is necessary in assessing antioxidant ability. Kulisic *et al.* (*32*) showed that single testing method was not sufficient to estimate the antioxidant ability of a studied sample. The combination of three methods (scavenging DPPH•, OH• and $O_2^{\bullet-}$ abilities) applied in this study can evaluate comprehensively the antioxidant ability. Preliminary experiments indicated that controlling enzymatic hydrolysis of bighead carp muscle proteins resulted in generation of peptides, which could scavenge the free radicals. After a short period of hydrolysis, the scavenging ability reached a maximum, and then it decreased. As an inexpensive and nonspecific protease, alcalase served the best for

Table 2. The prediction of ANN compared to the experimental results

	Input					Output								
	x_1	x_2	x_3	x_4	x_5	Scavenging ability on DPPH•			Scavenging ability on OH•			Scavenging ability on $O_2^{\bullet-}$		
						PR	OR	Error	PR	OR	Error	PR	OR	Error
1	3	4	4	3	2	87.68	87.49	0.19	67.32	67.06	0.26	41.44	41.21	0.23
2	3	4	3	2	1	77.01	76.86	0.15	65.62	66.13	0.51	49.57	49.46	0.11
3	4	3	4	1	2	68.04	68.59	0.55	68.53	68.09	0.44	37.43	37.09	0.34
4	4	4	2	4	1	59.45	60.04	0.59	72.55	71.98	0.57	40.75	41.03	0.28
	R^2							0.997			0.957			0.997
	E_r/%							1.35			7.60			1.94

PR: predicted response, OR: observed response

preparation of antioxidant peptides (data not shown). Additionally, the most important parameters were proved to be muscle/water ratio, hydrolysis time, E/S, pH and temperature. To evaluate the effect of the five variables, ANN was used to build hydrolysis model.

Developing ANN model usually consists of 3 steps: (*i*) choosing the ANN's construction. Theoretically, any 3-layer ANN could be applied to solve the simulation problem; (*ii*) training the ANN model with the training data. During this step, the optimal configuration of ANN such as learning coefficient, neutrons in the hidden, input and output layers are determined. It has been proved that the number of neutrons in the hidden layer should be confirmed during training. If the number is too large, the calculated speed will slow down, and if too small, the ANN will lose the accuracy. The same is valid for the learning coefficient. In this study, the number of neutrons in the hidden layer was 13 and learning coefficient was 0.03. The data in Table 1 show the input and output of learning samples used for BP network training; (*iii*) testing the model performance of trained ANN models using another set of data independent from the training data. In this study, 3 5-13-1 BP networks were used. Each neuron received information through input connections, then processed the information and finally produced the output that was distributed *via* output connection. After adjusting these weights by minimizing a non-linear error function, it achieved the goal (the mean-squared error between the predicted and experimental value equals to 10^{-3}) through 7058, 7983, and 8359 epochs.

To verify the forecasting reliability, BP network was used to predict the result, which was compared to the experimental value (Table 2). The high correlation coefficient (R^2>0.957) and low mean relative error (E_r<7.6 %) indicted that the trained ANN could simulate the enzymatic hydrolysis with high reliability, and the prediction ability gained during the learning process was easily transferred into unknown data, contained in verifying sets. Since excellent modeling performances were obtained by using the verifying data outside those used for training, it demonstrates that the ANN models were accurate and could be employed in a further analysis.

Optimization of the controlled hydrolysis

From a practical point of view, it is important to predict the optimization conditions for the production of antioxidant peptides and evaluate the effect of the hydrolysis parameters on antioxidant activity. Preliminary experiments indicated that the most important factors affecting peptide production were E/S, hydrolysis time and muscle/water ratio. To better understand the relationship between the hydrolysis parameters and the scavenging capability, ANN was designed to investigate the combined effect of time×E/S, time×(muscle/water) on the scavenging ability, and the 3-dimensional surface curve was plotted in Figs. 2a–c.

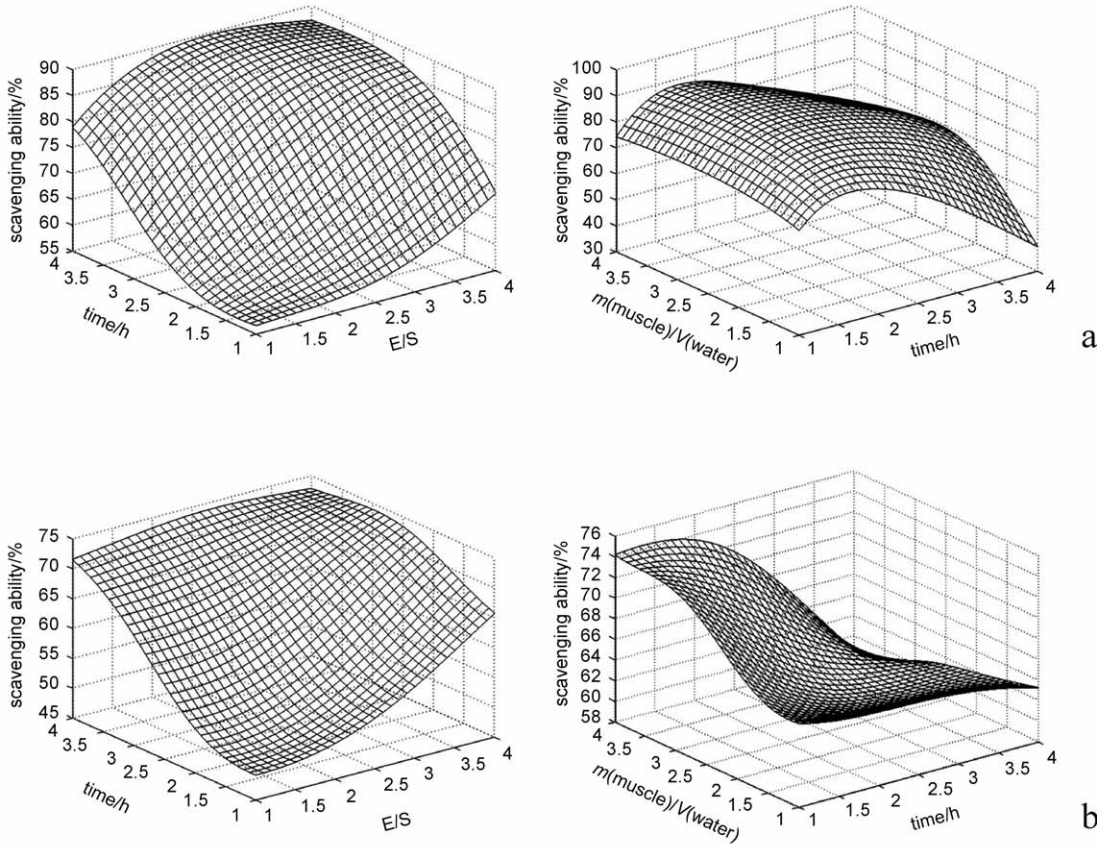

Fig. 2. The surface curve of the combined effect of time×enzyme/substrate, time×(muscle/water) on the scavenging free radicals ability of the hydrolysate (a on DPPH$^{\bullet}$, b on OH$^{\bullet}$, c on $O_2^{\bullet-}$)

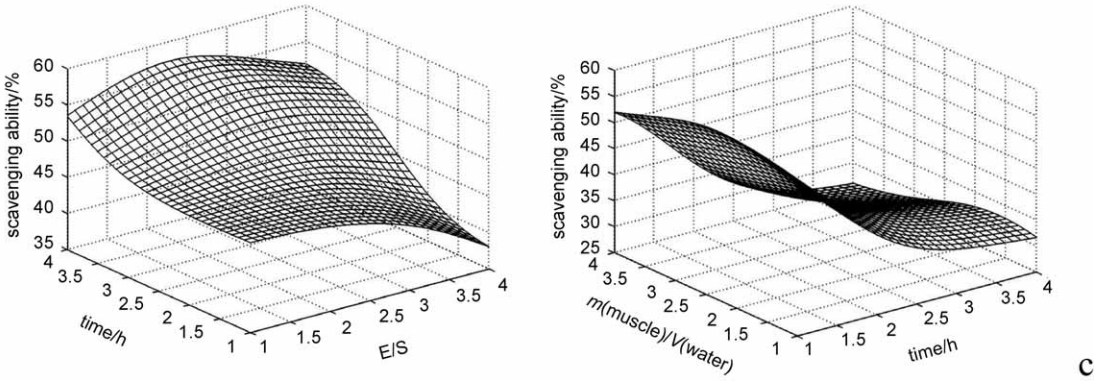

Fig. 2. Continued

From the surface curve, it was noticed that E/S, hydrolysis time and muscle/water affected the antioxidant ability of hydrolysates and exhibited interactive effects. Through the Matlab computation, the optimum operating conditions obtained from the ANN model were reported as: the hydrolysate A (HA), most effectively scavenging DPPH$^{\bullet}$; the hydrolysate B (HB), most effectively scavenging OH$^{\bullet}$; and the hydrolysate C (HC), most effectively scavenging $O_2^{\bullet-}$, were hydrolyzed for 4.8 h with an alcalase activity of 4.8 AU/kg, for 6 h with 3.84 AU/kg and for 4.3 h with 4.8 AU/kg, at pH=7.5 and 60 °C. Their respective muscle/water ratios were 1:1.9, 1:1.4 and 1:1. To test the model, new bighead carp hydrolysates were produced at optimal conditions predicted, and the scavenging abilities (HA on DPPH$^{\bullet}$, HB on OH$^{\bullet}$ and HC on $O_2^{\bullet-}$) were measured (Table 3). The errors between prediction values and experimental values were low (<2.25), which indicted that the results predicted by ANN were satisfactory and correlated well with the experimental values. Thus, the model based on ANN could offer a stable response in predicting the optimum production conditions. In addition, three hydrolysates showed stronger scavenging abilities compared to that of 0.04 mg/mL TBHQ, but lower than those of Vc at 0.2 and 2

Table 3. Values predicted by ANN compared to experimental results at the optimal hydrolysis conditions

Scavenging ability/%	PR	OR	Error
HA on DPPH$^{\bullet}$	90.32	91.80	1.48
HB on OH$^{\bullet}$	75.48	77.73	2.25
HC on $O_2^{\bullet-}$	57.27	58.05	0.78

PR: predicted response, OR: observed response

Table 4. Amino acid composition of bighead carp protein and bighead carp protein hydrolysate

Amino acid	Composition/%			
	Bighead carp proteins	Hydrolysate A	Hydrolysate B	Hydrolysate C
Aspartic acid + asparagine	10.46	8.45	9.14	9.51
Glutamine acid + glutamine	16.67	17.44	19.34	15.71
Serine	4.22	3.51	3.41	3.87
Glycine	4.09	4.07	3.80	4.35
Histidine	4.34	3.86	3.46	4.00
Arginine	6.40	5.34	5.07	5.76
Threonine	4.68	4.06	3.84	4.49
Alanine	4.99	4.31	4.10	4.67
Proline	3.77	3.66	3.43	3.99
Tyrosine	3.15	3.57	3.32	3.35
Valine	3.65	4.11	3.70	3.59
Methionine	2.38	3.73	3.80	3.78
Cysteine	0.27	0.19	0.20	0.22
Isoleucine	3.00	2.49	2.34	2.55
Leucine	5.86	4.96	4.47	5.16
Tryptophan	10.27	13.22	13.97	12.52
Phenylalanine	4.03	3.99	3.79	3.74
Lysine	7.76	9.05	8.80	8.74
Total	100.00	100.00	100.00	100.00

mg/mL (Table 1). It indicated that hydrolysates derived from bighead carp muscle protein may be used as natural antioxidants in food industry.

Property of bighead carp hydrolysates

The DH of hydrolysates A, B and C were 18.0, 20.0 and 17.3 %, while their PCL were 5.5, 5.0 and 5.8, respectively. The results indicated that three hydrolysates consisted mainly of short peptides that were composed of 5-6 amino acid residues, and proved that the length of the peptides could affect the antioxidant ability (*33*). This was in agreement with previous report in which the most of antioxidant peptides were composed of 5-16 amino acid residues (*34*). For hydrolysates A, B and C, high nitrogen recovery was achieved, with 76.0, 77.5 and 74.3 %, respectively. As shown in Table 4, the amino acid profiles of the hydrolysates were similar to those of the original bighead carp protein, except for methionine, tryptophan and lysine, which increased during hydrolysis. The PER values of hydrolysate A, B and C were 1.12, 0.92 and 1.53, respectively, as compared to 1.74 for the starting bighead carp proteins, which indicated that hydrolysis could release the antioxidant peptides from bighead carp protein while it reduced nutritional value, which is in agreement with other report (*6*). However, the changes in the antioxidant ability and nutritional value of the original bighead carp proteins by the hydrolysis need to be investigated further.

Conclusion

This is the first time that ANN has been applied to build hydrolysis model for optimizing the production of peptides. The model provided a quality prediction for the hydrolysis variables (pH, temperature, E/S, hydrolysis time and muscle/water ratio) in terms of free radical scavenging ability and optimized production conditions. ANN, a convenient and cheap tool, can be promising in modeling the controlled hydrolysis and predicting the biological properties of these peptides.

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References

1. I. Gill, R. López-Fadiño, X. Jorba, E.N. Vulfson, Biological active peptides and enzymatic approach to their production, *Enzyme Microb. Technol. 18* (1996) 162–183.
2. C.U. Carlsen, K.T. Rasmussen, K.K. Kjeldsen, P. Westergaard, L.H. Skibsted, Pro- and antioxidative activity of protein fractions from pork (*longissimus dorsi*), *Eur. Food Res. Technol. 217* (2003) 195–200.
3. S.Y. Jun, P.J. Park, W.K. Jung, S.K. Kim, Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (*Limanda aspera*) frame protein, *Eur. Food Res. Technol. 219* (2004) 20–26.
4. J.Y. Je, P.J. Park, S.K. Kim, Antioxidant activity of a peptide isolated from Alaska pollock frame protein hydrolysate, *Food Res. Int. 38* (2005) 45–50.
5. A.F. Morato, R.L. Carreira, R.G. Junqueira, Optimization of casein hydrolysis for obtaining high contents of small peptides: Using of subtillisin and trypsin, *J. Food Compos. Anal. 13* (2000) 843–857.
6. L.M.B. Candido, V.C. Sgarbieri, Enzymatic hydrolysis of Nile tilapia (*Oreochromus niloticus*) myofibrillar proteins: Effects on nutritional and hydrophilic properties, *J. Sci. Food Agric. 83* (2003) 937–944.
7. H.G. Kristinsson, B.A. Rasco, Biochemical and functional properties of Atlantic salmon (*Salmo salar*) muscle proteins hydrolyzed with various alkaline proteases, *J. Agric. Food Chem. 48* (2000) 657–666.
8. P.J. Bechtel, Properties of different fish processing by-products from pollock, cod and salmon, *J. Food Process. Preserv. 27* (2003) 101–106.
9. C. Ven, H. Gruppen, D.B.A. Bont, A.G.J. Voragen, Optimisation of the angiotensin converting enzyme inhibition by whey protein hydrolysates using response surface methodology, *Int. Dairy J. 12* (2002) 813–820.
10. S. Sathivel, P.J. Bechtel, J. Babbitt, S. Smiley, C. Crapo, K.D. Reppond, W. Prinyawiwatkul, Biochemical and functional properties of herring (*Clupea harregus*) by product hydrolysates, *Food Chem. Toxicol. 68* (2003) 2196–2200.
11. C.R. Chen, H.S. Ramaswamy, Analysis of critical control points in deviant thermal process using artificial neural networks, *J. Food Eng. 57* (2003) 225–235.
12. S. Haykin: *Neural Networks: A Comprehensive Foundation*, Prentice-Hall Inc., Hamilton, Ontario, Canada (2001) pp. 1–5.
13. S.I. Cho, S.C. Kim, Neural network modeling and fuzzy control of baking process, *T. ASAE, 41* (1998) 3548–3561.
14. W. Kaminski, P. Strumillo, E. Romczak, Neurocomputing approaches to modeling of drying process dynamics, *Dry. Technol. 16* (1998) 967–992.
15. J.S. Torrecilla, L. Otero, P.D. Sanz, A neural network approach for thermal/pressure food processing, *J. Food Eng. 62* (2004) 89–95.
16. M.A. Razavi, A. Mortazavi, M. Mousavi, Dynamic modelling of ilk ultrafiltration by artificial neural network, *J. Membr. Sci. 220* (2003) 47–58.
17. J. Ray, P. Kumar, R. Banerjee, Optimization of culture parameters for extracellular protease production from a newly isolated *Pseudomonas* sp. using response surface and artificial neural network models, *Process Biochem. 39* (2004) 2193–2198.
18. K.M. Desai, B.K. Vaidya, R.S. Singhal, Using of an artificial neural network in modeling yeast biomass and yield of β–glucan, *Process Biochem. 40* (2005) 1617–1626.
19. R.S. Parmar, R.W. McClendon, G. Hoogenboom, Estimation of aflatoxin contamination in preharvest peanuts using neural network, *T. ASAE, 40* (1997) 809–813.
20. M. Hajmeer, I. Basheer, Comparison of logistic regression and neural network based classifiers for bacterial growth, *Food Microbiol. 20* (2003) 43–55.
21. G.B. Quaglia, E. Orban, Influence of enzymatic hydrolysis on structure and emulsifying properties of sardine (*Sardine pilchardus*) protein hydrolysates, *J. Food Sci. 55* (1990) 1571–1573.
22. H.C. Wu, H.M. Chen, C.Y. Shiau, Free amino acid and peptides as related to antioxidant properties in protein hydrolysates of mackerel, *Food Res. Int. 36* (2003) 949–959.
23. B. Halliwell, J.M. Gutteridge, O.I. Aruoma, The deoxyribose method: A simple »test-tube« assay for determination of rate constants for reactions of hydroxyl radicals, *Anal. Biochem. 165* (1987) 215–219.
24. S. Marklund, G. Marklund, Involvement of the superoxide anion radicals in the autoxidation of pyrogallol and a con-

venient assay for superoxide dismutase, *Eur. J. Biochem. 47* (1974) 469–474.

25. Official Methods of Analysis, Association of Official Agricultural Chemists (AOAC), Washington DC, USA (1990).
26. M.L. Mahmoud, W.I. Malone, C.T. Cordle, Enzymatic hydrolysis of casein: Effect of degree of hydrolysis on antigenicity and physical properties, *J. Food Sci. 57* (1992) 1223–1229.
27. J. Adler-Nissen: *Enzymatic Hydrolysis of Food Proteins*, Elsevier Applied Science Publishers, New York, USA (1986) pp. 13–14.
28. S. Benjakul, M.T. Morrissey, Protein hydrolysates from Pacific Whiting solid wastes, *J. Agric. Food Chem. 45* (1997) 3423–3430.
29. Y.B. Lee, J.G. Elliot, D.A. Richansrud, E.C. Mugberg, Predicting protein efficiency ratio by the chemical determinations of connective tissue content in meat, *J. Food Sci. 43* (1978) 1359–1362.
30. R.H. Alsmeyer, A.E. Cunningham, M.L. Happich, Equations prediction PER from amino acid analysis, *Food Technol. 28* (1974) 34–40.
31. C.R. Chen, H.S. Ramaswamy, Modeling and optimization of variable retort temperature thermal processing using coupled neural networks and genetic algorithms, *J. Food Eng. 53* (2002) 209–220.
32. T. Kulisic, A. Radonic, V. Katalinic, M. Milos, Use of different methods for testing antioxidant activity of oregano essential oil, *Food Chem. 85* (2004) 633–640.
33. H.M. Chen, K. Muramoto, F. Yamauchi, K. Fujimoto, K. Nokihara, Antioxidant properties of histidine-containing peptides designed from peptide fragment found in the digests of a soybean protein, *J. Agric. Food Chem. 46* (1998) 49–53.
34. H.M. Chen, K. Muramoto, F. Yamauchi, Structural analysis of antioxidative peptides from soybean beta-conglycinin, *J. Agric. Food Chem. 43* (1995) 574–578.