Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 110 (2008) 294–300
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Optimization of tyrosinase inhibition activity of ultrasonic-extracted polysaccharides from longan fruit pericarp

Bao Yang [a], Mouming Zhao [b], Yueming Jiang [a,*]

[a] South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, PR China
[b] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, PR China

Received 3 October 2007; received in revised form 30 January 2008; accepted 31 January 2008

Abstract

Various ultrasonic conditions were employed to prepare polysaccharides from longan fruit pericarp (PLFP) and the Lineweaver–Burk equation was then used to determine the effect of PLFP on inhibition of tyrosinase activity. This result showed that PLFP acted as a non-competitive inhibitor of tyrosinase. The highest slope was observed for ultrasonic extraction, followed by the hot-water extraction, suggesting that the ultrasonic treatment of PLFP increased the inhibition of tyrosinase activity. Furthermore, a multilayer feed-forward neural network trained with an error back-propagation algorithm was used to evaluate the effects of ultrasonic power, time and temperature on the slope value. The trained network gave a regression coefficient (R^2) of 0.98 and a mean squared error (MSE) of 0.58, implying a good agreement between the predicted value and the actual value of the slope, and confirmed a good generalization of the network. Based on the artificial neural network-genetic algorithm, the optimal ultrasonic extraction conditions to obtain the highest slope value (154.1) were determined to be 120 W, 12 min and 57 °C. Application of response surface plots showed the slope value as a function of every two factors under various ultrasonic extraction conditions, which can be observed directly. Therefore, the artificial neural network provided a model with high performance and indicated the non-linear nature of the relation between ultrasonic conditions and slope value.

Keywords: Longan; Polysaccharide; Ultrasonic extraction; Artificial neural network-genetic algorithm; Tyrosinase

1. Introduction

Longan (*Dimocarpus longan* Lour.) is an important fruit in Southeast Asia (Jiang, Zhang, Joyce, & Ketsa, 2002) and longan fruit pericarp has been used in China for thousands of years as a traditional medicine. Unfortunately, the constituents in longan fruit pericarp tissues and their biological activities are still unclear. Our previous work has found a significant amount of polysaccharides present in longan fruit pericarp tissue. A great deal of attention has been paid to polysaccharides for their unique biological, chemical and physical properties (Schepetkin & Quinn, 2006). Polysaccharides contribute to the development of important therapeutic drugs used currently in modern medicine and cosmetics (Li, Zhou, & Han, 2006). Recently, Rout and Banerjee (2007) have reported that polysaccharides show a good inhibition activity of tyrosinase, which might be helpful to extend the use of polysaccharides in modern medicine and cosmetics.

Tyrosinase (EC 1.14.18.1) is a multifunctional enzyme that catalyzes both the hydroxylation of monophenols such as tyrosine to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones. Meanwhile, the enzyme is widely distributed in organisms and plays an important role in melanin production (Park et al., 2005). Alterations in melanogenesis may be responsible for a part of clinical and histopathological features unique to malignant melanoma, a cancer with a fast increase of incidence (Baurin, Arnoult, Scior, Do, & Bernard, 2002). Therefore, tyrosinase inhibitors may be clinically useful for the treatment of skin cancer

* Corresponding author. Tel.: +86 20 37252525; fax: +86 20 37252831.
E-mail address: ymjiang@scbg.ac.cn (Y. Jiang).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.01.067

and some dermatological disorders associated with melanin hyperpigmentation and are important in cosmetics for whitening and depigmentation after sunburn (Shaheen et al., 2005).

Ultrasonic treatment has been employed for preparing polysaccharides from different plant materials in recent years (Hromadkova & Ebringerova, 2003). The enhanced extraction by ultrasonic treatment is mainly attributed to its mechanical effects, which greatly facilitate mass transfer between immiscible phases through a super agitation, especially at low frequency (Vinatoru et al., 1997). However, degradation of polysaccharides by ultrasonic treatment can occur (Zhou & Ma, 2006) and this could lead to changes in the structure and other characteristics of polysaccharides (Mislovicova, Masarova, Bendzalova, Soltes, & Machova, 2000). Because the structure and molecular weight of polysaccharides are related to the bioactivities, such as regulatory capacity of enzyme activity and radical scavenging ability (Zhang, Zhang, Cheung, & Ooi, 2004), it is interesting to investigate the role of ultrasonic treatment in polysaccharide bioactivities.

The artificial neural network as an influential tool of artificial intelligence has been in existence for more than 50 years and has been applied for engineering fields (Suah, Ahmad, & Taib, 2003). Based on the connection pattern, the artificial neural network can be grouped into two categories as the feed-forward network and feedback networks. The development of error back-propagation learning algorithms for determining weight in a multilayer perceptron has made multilayer feed-forward networks very popular among researchers and users of neural networks (Srecnik, Debeljak, Cerjan-Stefanovic, Novic, & Bolanca, 2002). Genetic algorithms are probabilistic-search techniques based on the principle of biological evolution and have been widely employed in the optimization of manufacturing and industrial engineering processes (Sette, Boullart, & Van Langenhove, 1998).

In this present study, ultrasonic technique was employed to extract PLFP while a multilayer feed-forward neural network trained with an error back-propagation algorithm was used to further evaluate the effects of PLFP, prepared by various ultrasonic power, time and temperature, on the inhibition of tyrosinase activity. Genetic algorithms were also used to optimize the ultrasonic conditions for preparing PLFP to obtain the highest inhibition of tyrosinase activity.

2. Materials and methods

2.1. Plant materials

Longan (*Dimocarpus longan* Lour. cv. Shixia) is a non-climateric fruit, and will not continue to ripen once removed from the tree. Consequently, fruit must be harvested when their skins become yellow-brown and their flesh reaches the optimal eating quality. In this study, fresh longan fruits at a commercial maturity standard were purchased from a commercial market in Guangzhou. Fruits were selected for uniformity of shape and yellow colour.

2.2. Chemicals

L-tyrosine and tyrosinase with an activity of 1000 units/mg were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Glucose, phenol and sulfuric acid were obtained from Guangzhou Reagent Co. (Guangzhou, China). All other chemicals used were of analytical grade.

2.3. Extraction and quantification of PLFP

Four grams of longan fruit pericarp and 100 ml of distilled water were used for each extraction. The extraction was performed using an ultrasonic cleaner (SB-5200DTD, Xinzhi Biotech Co., Ningbo, China, 40 kHz), using selected ultrasonic power and temperature for various durations. The extract was then filtered through a 9-cm filter paper. The filtrate was concentrated to 25 ml using a rotary evaporator (BC-R203, Shanghai Biochemical Equipment Co., Shanghai, China) at 65 °C under vacuum. The proteins in the extract were removed by Sevag reagent (Navarini et al., 1999). Sevag reagent (100 ml:80 ml of $CHCl_3$ and 20 ml of butanol) was added to the concentrated extract. The bulk was shaken for 20 min at 25 °C. After centrifugation at 3000*g* for 20 min, the supernatant was collected and subjected to this step for four times. After removal of the Sevag reagent, 100 ml of anhydrate ethanol was added before the mixture was maintained overnight at 4 °C to precipitate polysaccharides. PLFP was obtained by centrifugation at 3860*g* for 15 min.

Hot-water extraction was also employed for PLFP preparation as a control according to the method of Yang et al. (2006). Longan fruit pericarp tissues (4 g) were extracted for 1 h with 100 ml of distilled water at 60 °C and then filtered. The subsequent extraction of PLFP was the same as the above-mentioned procedures.

The polysaccharide content in PLFP was determined by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), used glucose as standard, and the results were then expressed as glucose equivalents.

2.4. Assay of inhibition of tyrosinase activity

Inhibition of tyrosinase activity was tested according to the method of Rout and Banerjee (2007) with minor modifications. L-tyrosine solution (4 ml) at 0.1, 0.2, 0.3 or 0.4 mg/ml, dissolved previously in 20 mM phosphate buffer (pH 6.8), was added to 1 ml of 50 μg/ml PLFP solutions. After 20 min of incubation, 1 ml of mushroom tyrosinase (50 units/ml, dissolved in 20 mM phosphate buffer, pH 6.8) was added to the mixture solution. The absorbance was recorded for 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 min at 475 nm, respectively. The control was used with 1 ml of distilled water instead of PLFP sample. Lineweaver–Burk plot

was drawn following the following equation (Lineweaver & Burk, 1934).

$$\frac{1}{V} = \frac{K_m}{V_m} \times \frac{1}{S} + \frac{1}{V_m} \quad (1)$$

The inhibition mode was assayed by Lineweaver–Burk plot, and the study showed that PLFP acted as a non-competitive inhibitor. Therefore, the following non-competitive inhibition equation was employed to further analyse the effect of PLFP.

$$\frac{1}{V} = \frac{K_s \times \left(1 + \frac{1}{K_I}\right)}{V_m} \times \frac{1}{S} + \frac{1}{V_m} \times \left(1 + \frac{1}{K_I}\right) \quad (2)$$

where I was the concentration of inhibitor while K_I was the inhibition constant. A higher slope meant a better inhibition activity.

2.5. Modeling of neural network

The multilayer feed-forward neural network has proven to be an excellent universal approximator of non-linear functions. The architecture is a fully connected structure. Each neuron is connected to all the neurons in the subsequent layer (Dreyfus & Deryfus, 2003). In the present study, a feed-forward neural network trained with an error back-propagation algorithm was employed using MATLAB (Version 6.5, Mathworks, Natick, MA) Neural Network Toolbox to model the slope value as a function of independent variables taken for ultrasonic treatments. The input parameters chosen in this study were ultrasonic power, time and temperature. The input data should be normalized within a proper range to avoid any numerical overflow. The output parameters were normalized between −1 and 1 in this work. The neurons in the input layer introduce the normalized input data to the hidden layer via weights. The neurons in the hidden layer sum up the weighted inputs to neurons, including bias as shown by Eq. (3). The weighted output is then activated using hyperbolic tangent sigmoid transfer function, as shown in Eq. (4). The output produced by the hidden layer becomes an input to output layer. The neurons in the output layer produce the output using linear transfer function (Eq. (5)).

$$\text{net} = \sum_{i=1}^{n} x_i w_i + \theta \quad (3)$$

where w_i (i =1, n) was the connection weight, θ was bias and x_i was the input data while *net* was the activation.

$$\text{Tansig (net)} = \frac{2}{1 + \exp(-2^{\times}\text{net})} - 1 \quad (4)$$

$$\text{Purelin } (x_i) = x_i \quad (5)$$

Supervised learning was used to train this network. The predicted output and desired output were compared with one another while the errors were calculated between the predicted output and actual output. An error back-propagation algorithm was used for adjusting the weights. It used a gradient descent approach, in which weights were changed in proportion to the negative of the error gradient. The training iterations were stopped when the validation error reached a set minimum (Desai, Vaidya, Singhal, & Bhagwat, 2005).

2.6. Optimization of ultrasonic treatment conditions

Genetic algorithms can solve well the linear and non-linear problems by exploring all regions of the state spaces and exploiting promising areas through selection, crossover and mutation operations applied to individuals in the population (Shen, Wang, & Li, 2007). Once the feed-forward neural network model was completely trained, genetic algorithms could be used to determine the optimization of ultrasonic extraction conditions for the highest slope value.

2.7. Statistical analysis

Data were expressed as the means of three replicate determinations. Statistical calculations by Microsoft Excell 2003 (Micrisoft, Seattle, WA, USA) were conducted to calculate the correlation.

3. Results and discussion

3.1. Effects of ultrasonic power, time and temperature on slope values

Melanogenesis is a physiological process resulting in the melanin synthesis while the epidermal hyperpigmentation, leading to various dermatological disorders such as melasma, freckles and age spots, might be related to alteration of melanogenesis (Hsu, Chang, Lu, & Chung, 2007). Tyrosinase is known to be a key enzyme for melanin biosynthesis (Godbole, Mojamdar, & Pal, 2006). In addition, tyrosinase is involved in browning of fruits and vegetables. Thus, the search of natural chemicals to inhibit tyrosinase activity is of great interest at present. Some of the known natural compounds, such as kojic acid (Cabanes, Chazarra, & Garcia-Carmona, 1994), catechin (No et al., 1999) and resveratrol (Bernard & Berthon, 2000), have been described for inhibition of tyrosinase activity. Kojic acid and catechin are competitive inhibitors of tyrosinase while resveratrol acts as a non-competitive inhibitor (Cabanes et al., 1994; No et al., 1999). In this study, PLFP inhibited tyrosinase activity in a non-competitive manner (Fig. 1), which was in agreement with the report of Rout and Banerjee (2007). All PLFP samples prepared by various ultrasonic extractions showed a higher slope value than those by the hot-water extraction, indicating that ultrasonic treatment could enhance the inhibition of tyrosinase activity. Figs. 2–4 further show the effects of ultrasonic power, time and temperature, and their interactions on the slope values. The non-linear nature of the relation between ultrasonic extraction conditions and the slope values could be

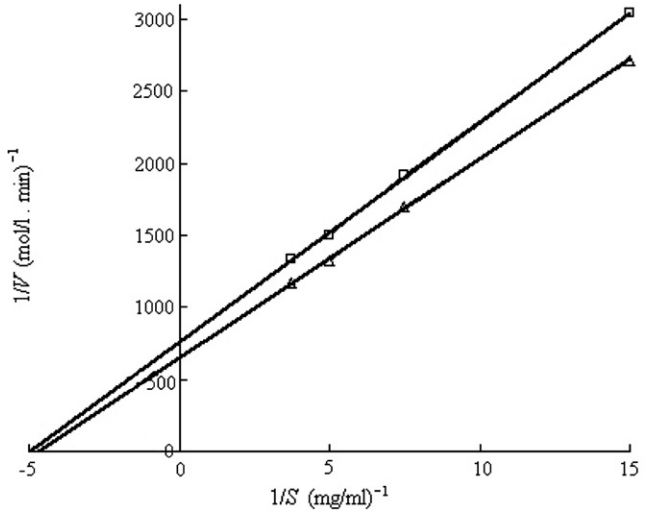

Fig. 1. Lineweaver–Burk plots showing $1/V$ of the tyrosinase versus $1/S$, with L-tyrosine as the substrate. (Δ) The tyrosinase reaction in the absence of inhibitor; (□) the tyrosinase reaction in the presence of PLFP prepared at 120 W and 57 °C for 12 min.

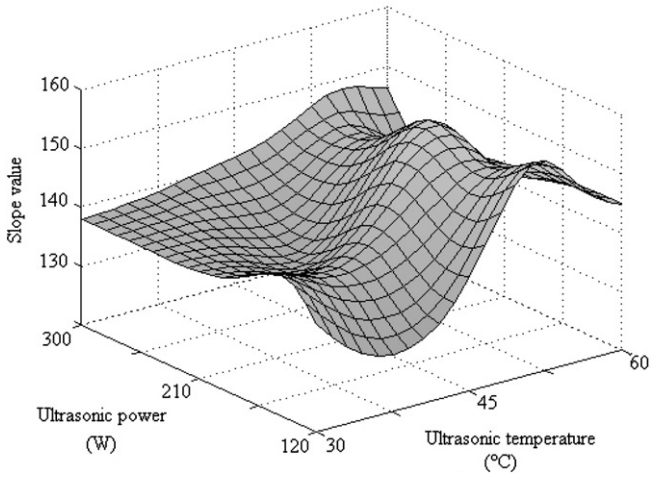

Fig. 3. Response surface plot showing the effects of ultrasonic power, temperature, and their interactions on the slope values. The ultrasonic temperature was kept constant at 45 °C.

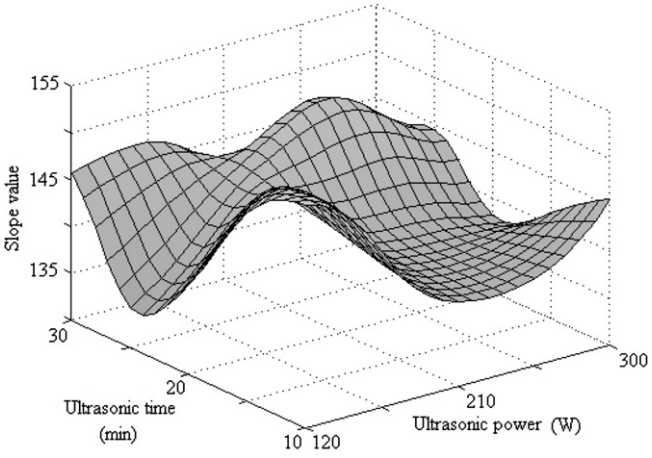

Fig. 2. Response surface plot showing the effects of ultrasonic power and time, and their interactions on the slope values. The ultrasonic time was kept constant for 20 min.

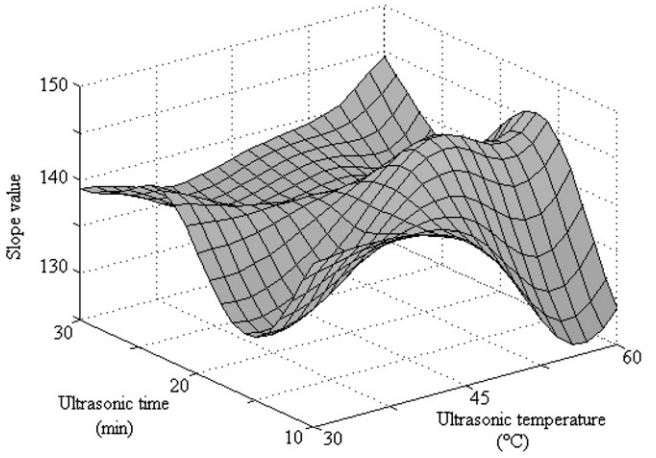

Fig. 4. Response surface plot showing the effects of ultrasonic temperature and time, and their interactions on the slope values. The ultrasonic power was kept constant at 210 W.

observed easily and directly from these figures. Each of these three factors exhibited a significant effect on the slope value. As shown in Fig. 2, the highest slope value could be obtained when ultrasonic power and ultrasonic time were close to the lower limits. Complicated interactions between two of three factors were also found. For example, when 10 min of ultrasonic duration and 45 °C of ultrasonic temperature were used the slope value decreased with increasing ultrasonic power to a certain value (139.8) and thereafter increased, but the slope value increased with increasing ultrasonic power to a certain value (145.9) and then decreased as 20 min of ultrasonic duration and 45 °C of ultrasonic temperature were applied (Fig. 2). The possible mechanism was due to the degradation effect of ultrasonic wave, which could modify the molecular weight, steric conformation and chemical structure of PLFP (Li, Guo, & Li, 2005; Sivakumar & Pandit, 2001). Szu, Zon, Schneerson, and Robbins (1986) have pointed out that ultrasonic treatment can depolymerize polysaccharides to a finite molecular weight. Meanwhile, the cleavage of the linkages between the repeating units of polysaccharides can be caused by ultrasonic treatment. It is well known that molecular weight and chemical structure of polysaccharides are highly related to their bioactivities (Wu, Zheng, Ning, & Yang, 2007). After ultrasonic treatment, an improved DPPH radical scavenging activity was observed for polysaccharides of longan fruit (Yang, Zhao, Shi, Yang, & Jiang, 2008). The polysaccharides isolated from sclerotia and mycelia of *Pleurotus tuber-regium* by ultrasonic treatment showed a different molecular profile and anti-tumour activity (Zhang et al., 2004). Therefore, degradation upon ultrasonic treatment was responsible for the change in the inhibition of tyrosinase activity of PLFP in this work.

PLFP, as a polysaccharide, consists of many monomers, each containing several hydroxyl groups. The inhibition of tyrosinase activity might be dependent on the hydroxyl group of the PLFP that could form a hydrogen bond to a site of the enzyme leading to a lower enzymatic activity. Song et al. (2006) reported that some tyrosinase inhibitors act through hydroxyl groups which bind with active site of tyrosinase resulting in steric hindrance or changed conformation. Furthermore, the antioxidant activity is another important mechanism for the inhibition of tyrosinase activity (Kubo, Chen, & Nihei, 2003). Plant polysaccharides are an important source of antioxidants which can show strong reducing power and free-radical scavenging activity (Kardosova & Machova, 2006). In our previous work, PLFP has been confirmed to be a strong radical scavenger (Yang et al., 2008). Therefore, it is possible that the products formed by tyrosinase oxidation can be reduced to the form of substrate by PLFP.

3.2. Evaluation of model predictability

R^2 was defined as the ratio of the explained variation to the total variation, which reflected the degree of fitness (Nath & Chattopadhyay, 2007). The model fits better the actual data when R^2 approaches unity (Sin, Yusof, Hamid, & Rahman, 2006). The network having minimum MSE and maximum R^2 was considered as the best neural network model (Izadifar & Jahromi, 2007). The performance of the artificial neural network was statistically analyzed by MSE and R^2 obtained as follows:

$$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}(y_i - y_{\mathrm{d}i})^2 \tag{6}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - y_{\mathrm{d}i})^3}{\sum_{i=1}^{n}(y_{\mathrm{d}i} - y_m)^2} \tag{7}$$

where n was the number of data while y_i was the predicted value by neural network model and $y_{\mathrm{d}i}$ was the actual value while y_m was the average of the actual values.

The incorporated network effects of three ultrasonic factors at a range were given below: ultrasonic power (120–300 W), duration (10–30 min) and temperature (30–60 °C). Table 1 listed the input matrix and slope values. To understand the generalization capacity of the network, 27 input values were divided into three sets, 19 values for training set and 4 values for validation and testing sets each. Fig. 5 shows the plots of the predicted value and the actual value of the slope as well as R^2 and MSE. The trained network gave R^2 of 0.98 and MSE of 0.58. The R^2 and MSE of training set, validation set and testing set were 0.99, 0.92 and 0.90 while the MSE of the training set, validation set and testing set were 0.47, 0.88 and 0.58, respectively. The network predicted the slope within a range of ±2.3% of the actual value. These data implied a good agreement between the predicted value of the neural network model and the actual value and confirmed a good generalization of the network.

Table 1
The predicted values of the neural network and the actual values of the slope of PLFP prepared under different ultrasonic extraction conditions

Experiment	Ultrasonic power (W)	Ultrasonic time (min)	Ultrasonic temperature (°C)	Slope value	
				Predicted value	Actual value
Training set					
1	120	10	30	148.5	148.6
2	120	10	45	153.9	153.9
3	120	10	60	153.0	152.6
4	120	20	30	137.9	138.4
5	120	20	45	139.6	139.6
6	120	20	60	145.3	144.9
7	120	30	60	145.7	145.7
8	210	10	30	142.8	142.6
9	210	10	60	128.4	128.9
10	210	20	45	141.4	141.2
11	210	20	60	142.4	142.8
12	210	30	30	138.7	138.2
13	210	30	45	139.1	138.3
14	210	30	60	144.1	144.4
15	300	10	30	151.1	152.9
16	300	10	45	146.5	146.8
17	300	10	60	141.6	141.0
18	300	20	30	137.9	137.9
19	300	20	45	138.8	140.6
Validation set					
20	210	20	30	138.4	138.1
21	300	20	60	145.2	146.2
22	300	30	30	138.0	138.9
23	300	30	60	145.8	144.2
Testing set					
24	120	30	30	140.2	140.5
25	120	30	45	145.0	145.4
26	210	10	45	140.9	140.3
27	300	30	45	142.2	140.9

Application of the artificial neural network-genetic algorithm indicated that the optimum ultrasonic extraction conditions for the highest slope value were determined to be 120 W, 12 min and 57 °C. The predicted value of the slope was 154.1. As the estimated optimal ultrasonic extraction conditions were used in the actual preparation

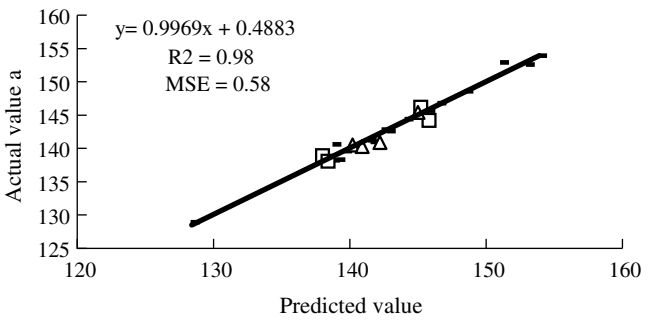

Fig. 5. Correlation between the predicted values of the neural network and the actual values of the slope. (—), Training set; (□) validation set and (△) testing set.

of the PLFP, the slope value was measured experimentally to be was 152.9 ± 1.6, which was not significantly different ($P > 0.05$) from the predicted value.

4. Conclusions

PLFP acted as a non-competitive inhibitor against tyrosinase. The PLFP prepared by ultrasonic extraction exhibited the highest slope value, followed by the hot-water extraction, which suggested that ultrasonic-extracted-PLFP increased the inhibition of tyrosinase activity. A multilayer feed-forward neural network trained with an error back-propagation algorithm was used to further assay the effects of ultrasonic power, time and temperature on the slope value. By comparison of the predicted values of the neural network model and the actual values of the slope, the trained network gave R^2 of 0.98 and MSE of 0.58, which implied a good agreement between the predicted value and the actual value, and confirmed a good generalization of the network on new data. Based on the combination of the artificial neural networks and genetic algorithms, the optimum ultrasonic extraction conditions to obtain the highest slope value were determined to be 120 W, 12 min and 57 °C. The slope value of the actual measurement under the optimum predicated ultrasonic extraction conditions was 152.9 ± 1.6, which was not significantly different ($P > 0.05$) to the predicted value (154.1). Thus, the artificial neural networks provided a model to search for the non-linear nature between ultrasonic extraction conditions and the slope value in a short-term experiment. However, more specific mechanism of the inhibition of tyrosinase activity by the ultrasonic-extracted PLFP needs to be further investigated.

Acknowledgements

The financial support provided by the National Natural Science Foundation of China (Grant Nos. 30425040 and 30700557), Postdoctor Science Foundation of China (No. 20070420802) and the Eleventh-five-year National Key Technology R&D Program (No. 2006BAD27B03 and 2006BAD27B04) was appreciated.

References

Baurin, N., Arnoult, E., Scior, T., Do, Q. T., & Bernard, P. (2002). Preliminary screening of some tropical plants for anti-tyrosinase activity. *Journal of Ethnopharmacology, 82*, 155–158.

Bernard, P., & Berthon, J. Y. (2000). Resveratrol: An original mechanism on tyrosinases inhibition. *International Journal of Cosmetic Sciences, 22*, 219–226.

Cabanes, J., Chazarra, S., & Garcia-Carmona, F. (1994). Kojic acid, a cosmetic skin whitening agent, is a slow-binding inhibitor of catecholase activity of tyrosinase. *Journal of Pharmacy and Pharmacology, 46*, 982–985.

Desai, K. M., Vaidya, B. K., Singhal, R. S., & Bhagwat, S. S. (2005). Use of an artificial neural network in modeling yeast biomass and yield of b-glucan. *Process Biochemistry, 40*, 1617–1626.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Dreyfus, C., & Deryfus, G. (2003). A machine learning approach to the estimation of the liquidus temperature of glass-forming oxide blends. *Journal of Non-crystalline Solids, 318*, 63–78.

Godbole, D., Mojamdar, M., & Pal, J. K. (2006). Increased level of p27 subunit of proteasomes and its co-localization with tyrosinase in amelanotic melanoma cells indicate its direct role in the regulation of melanin biosynthesis. *Cell Biology International, 30*, 895–902.

Hromadkova, Z., & Ebringerova, A. (2003). Ultrasonic extraction of plant materials – investigation of hemicellulose release from buckwheat hulls. *Ultrasonics Sonochemistry, 10*, 127–133.

Hsu, C. K., Chang, C. T., Lu, H. Y., & Chung, Y. C. (2007). Inhibitory effects of the water extracts of *Lavendula* sp. on mushroom tyrosinase activity. *Food Chemistry, 105*, 1099–1105.

Izadifar, M., & Jahromi, M. Z. (2007). Application of genetic algorithm for optimization of vegetable oil hydrogenation process. *Journal of Food Engineering, 78*, 1–8.

Jiang, Y. M., Zhang, Z. Q., Joyce, D. C., & Ketsa, S. (2002). Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour. *Postharvest Biology and Technology, 26*, 241–252.

Li, J., Guo, S., & Li, X. (2005). Degradation kinetics of polystyrene and EPDM melts under ultrasonic irradiation. *Polymer Degradation and Stability, 89*, 6–14.

Li, X. L., Zhou, A. G., & Han, Y. (2006). Anti-oxidant and anti-microorganism activities of purification polysaccharide from *Lygodium japonicum in vitro*. *Carbohydrate Polymers, 66*, 34–42.

Kardosova, A., & Machova, V. (2006). Antioxidant activity of medicinal plant polysaccharides. *Fitoterapia, 77*, 367–373.

Kubo, I., Chen, Q. X., & Nihei, K. I. (2003). Molecular design of antibrowning agents: Antioxidative tyrosinase inhibitors. *Food Chemistry, 81*, 241–247.

Lineweaver, H., & Burk, D. (1934). The determination of enzyme dissociation constants. *Journal of the American Chemical Society, 57*, 666–685.

Mislovicova, D., Masarova, J., Bendzalova, K., Soltes, L., & Machova, E. (2000). Sonication of chitin-glucan, preparation of water-soluble fractions and characterization by HPLC. *Ultrasonics Sonochemistry, 7*, 63–68.

Nath, A., & Chattopadhyay, P. K. (2007). Optimization of oven toasting for improving crispness and other quality attributes of ready to eat potato-soy snack using response surface methodology. *Journal of Food Engineering, 80*, 1282–1292.

Navarini, L., Gilli, R., Gombac, V., Abatangelo, A., Bosco, M., & Toffanin, R. (1999). Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: Isolation and characterization. *Carbohydrate Polymers, 40*, 71–81.

No, J. K., Soung, D. Y., Kim, Y. J., Shim, K. H., Jun, Y. S., Rhee, S. H., et al. (1999). Inhibition of tyrosinase by green tea components. *Life Sciences, 65*, 241–246.

Park, K. H., Park, Y. D., Lee, J. R., Hahn, H. S., Lee, S. L., Bae, C. D., et al. (2005). Inhibition kinetics of mushroom tyrosinase by copper-chelating ammonium tetrathiomolybdate. *Biochimica et Biophysica Acta, 1726*, 115–120.

Rout, S., & Banerjee, R. (2007). Free radical scavenging, anti-glycation and tyrosinase inhibition properties of a polysaccharide fraction isolated from the rind from *Punica granatum*. *Bioresource Technology, 98*, 3159–3163.

Schepetkin, I. A., & Quinn, M. T. (2006). Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. *International Immunopharmacology, 6*, 317–333.

Sette, S., Boullart, L., & Van Langenhove, L. (1998). Using genetic algorithms to design a control strategy of an industrial process. *Control Engineering Practice, 6*, 523–527.

Shaheen, F., Ahmad, M., Khan, M. T. H., Jalil, S., Ejaz, A., Sultankhodjaev, M. N., et al. (2005). Alkaloids of *Aconitum laeve* and their anti-inflammatory, antioxidant and tyrosinase inhibition activities. *Phytochemistry, 66*, 935–940.

Shen, C., Wang, L., & Li, Q. (2007). Optimization of injection molding process parameters using combination of artificial neural network and genetic algorithm method. *Journal of Materials Processing Technology, 183*, 412–418.

Sivakumar, M., & Pandit, A. B. (2001). Ultrasound enhanced degradation of Rhodamine B: Optimization with power density. *Ultrasonics Sonochemistry, 8*, 233–240.

Sin, H. N., Yusof, S., Hamid, N. S. A., & Rahman, R. A. (2006). Optimization of enzymatic clarification of sapodilla juice using response surface methodology. *Journal of Food Engineering, 73*, 313–319.

Song, K. K., Huang, H., Han, P., Zhang, C. L., Shi, Y., & Chen, Q. X. (2006). Inhibitory effects of cis- and trans-isomers of 3,5-dihydroxystilbene on the activity of mushroom tyrosinase. *Biochemical and Biophysical Research Communications, 342*, 1147–1151.

Srecnik, G., Debeljak, Z., Cerjan-Stefanovic, S., Novic, M., & Bolanca, T. (2002). Optimization of artificial neural networks used for retention modelling in ion chromatography. *Journal of Chromatography A, 973*, 47–59.

Suah, F. B. M., Ahmad, M., & Taib, M. N. (2003). Optimisation of the range of an optical fibre pH sensor using feed-forward artificial neural network. *Sensors and Actuators B: Chemical, 90*, 175–181.

Szu, S. C., Zon, G., Schneerson, R., & Robbins, J. B. (1986). Ultrasonic irradiation of bacterial polysaccharides, characterization of the depolymerized products and some applications of the process. *Carbohydrate Research, 152*, 7–20.

Vinatoru, M., Toma, M., Radu, O., Filip, P. I., Lazurca, D., & Mason, T. J. (1997). The use of ultrasound for the extraction of bioactive principles from plant materials. *Ultrasonics Sonochemistry, 4*, 135–139.

Wu, Q., Zheng, C., Ning, Z. X., & Yang, B. (2007). Modification of low molecular weight polysaccharides from *Tremella fuciformis* and their antioxidant activity *in vitro*. *International Journal of Molecular Sciences, 8*, 670–679.

Yang, B., Wang, J. S., Zhao, M. M., Liu, Y., Wang, W., & Jiang, Y. M. (2006). Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis* Sonn.) fruit in relation to their antioxidant activities. *Carbohydrate Research, 341*, 634–638.

Yang, B., Zhao, M. M., Shi, J., Yang, N., & Jiang, Y. M. (2008). Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. *Food Chemistry, 106*, 685–690.

Zhang, M., Zhang, L., Cheung, P. C. K., & Ooi, V. E. C. (2004). Molecular weight and anti-tumor activity of the water-soluble polysaccharides isolated by hot water and ultrasonic treatment from the sclerotia and mycelia of *Pleurotus tuber-regium*. *Carbohydrate Polymers, 56*, 123–128.

Zhou, C., & Ma, H. (2006). Ultrasonic degradation of polysaccharide from a red algae (*Porphyra yezoensis*). *Journal of Agricultural and Food Chemistry, 54*, 2223–2228.