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

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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

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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