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Anti-glycated activity prediction of polysaccharides from two guava fruits using artificial neural networks

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ABSTRACT

High-efficiency ultrasonic treatment was used to extract the polysaccharides of *Psidium guajava* (PPG) and *Psidium littorale* (PPL). The aims of this study were to compare polysaccharide activities from these two guavas, as well as to investigate the relationship between ultrasonic conditions and anti-glycated activity. A mathematical model of anti-glycated activity was constructed with the artificial neural network (ANN) toolbox of MATLAB software. Response surface plots showed the correlation between ultrasonic conditions and bioactivity. The optimal ultrasonic conditions of PPL for the highest anti-glycated activity were predicted to be 256 W, 60 °C, and 12 min, and the predicted activity was 42.2%. The predicted highest anti-glycated activity of PPG was 27.2% under its optimal predicted ultrasonic condition. The experimental result showed that PPG and PPL possessed anti-glycated and antioxidant activities, and those of PPL were greater. The experimental data also indicated that ANN had good prediction and optimization capability.

1. Introduction

Guava is a tropical fruit that consists of a fleshy pericarp, a seed cavity with white fleshy pulp, and numerous small seeds. It has been used as a functional food and folk medicine for the adjuvant treatment of diabetes mellitus in China. However, the bioactive constituents in guava fruit remain unknown.

Psidium guajava Linn. is a well-known type of guava tree that is a common plant in South China and is widely planted in tropical areas. Different parts of the plant are used to treat various human ailments, such as wounds, ulcers, bowels, and cholera (Gutierrez, Mitchell, & Solis, 2008). Pharmacological investigations indicate that its bark, fruit, seeds, and leaves possess anti-diabetic, anti-bacterial, anti-hyperglycemic, anti-hyperlipidemic, anti-oxidant, anti-diarrheal, anti-inflammatory, anti-atherogenic, and analgesic activities (Alonso-Castro et al., 2012; Choi et al., 2012; Deguchi & Miyazaki, 2010; Martinez et al., 2012; Semenya and Maroyi, 2012; Shen, Cheng, & Wu, 2008; Soman, Rajamanickam, Rauf, & Indira, 2013; Tavares et al., 2012; York, Wet, & Vuurenb, 2011; Yoshitomi, Guo, Liu, & Gao, 2012). *Psidium littorale* Raddi (syn. *P. cattleianum* Sabine) (Lapčík et al., 2005) is another member of the *Psidium* genus but have rarely been studied. Just a few words were used to describe this guava in the Flora of China (Chinese Academy of Sciences-Flora Repubulicae Popularis Sinicae Editorial Board, 2004). *P. littorale* Raddi is called "strawberry guava" due to its pink pulp. Because they belong to the same genus, *P. littorale* (PL) may share some common properties with *P. guajava* (PG). Therefore, it is meaningful to study and compare both guava species.

Polysaccharides are important functional ingredients of food and medicine formulations (Schepetkin & Quinn, 2006), and their unique bioactivities have garnered a great deal of attention in recent years (Zong, Cao, & Wang, 2012). The results of our previous experiments suggest that polysaccharides in guava fruits contribute to their clinical utility.

Ultrasonic treatment has been shown to have high extracting efficiency and has been used to prepare natural polysaccharides for decades (Hromádková & Ebringerová, 2003). The mechanism of ultrasonic enhancement varies depending on ultrasonic power, temperature, and time. This can lead to the changes in polysaccharide structure and the enhancement of its bioactivity (Chen et al., 2012). Anti-glycated activity and radical scavenging ability are certainly linked with polysaccharide structure and molecular mass (Yang, Zhao, & Jiang, 2009). This study aimed to determine the relationship between ultrasonic conditions (selected power, temperature and time) and anti-glycated polysaccharide activity.

An artificial neural network (ANN) is composed of a set of virtual/artificial neurons organized in interconnected layers (Cabrera & Prieto, 2010). Each neuron has a specific weight in information processing, and the optimal weights are calculated with available pairs of input and output data constituting the training set. Using these pairs, the ANN is able to minimize output error, modifying weights as required. While two of these layers are connected to the

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"outside world" (input layer, where data is presented and output layer, where a prediction value is obtained), the remainder (hidden layers) are defined by neurons connected to each other that intra-layer connections. Due to their inherent capabilities, ANNs can be successfully used for function approximation, pattern classification, associative memory, and new pattern generation. The applications of ANNs spread over a very large set of fields, including medicine, neurology, chemistry, mathematics, engineering, economics, meteorology, psychology, robotics, and transportation (Chau, 2006; Cimpoiu, Cristea, Hosu, Sandru, & Seserman, 2011; Levine, 2007; Lisboa, 2002; Lisboa & Taktak, 2006).

To our knowledge, there has been no report on bioactivity prediction of polysaccharides from guava fruits using ANNs. In this study, we predicted the anti-glycated activity of polysaccharides with mathematical models. The model was built with combinations of independent variables (ultrasonic power, time, and temperature) and dependent variables (anti-glycated activity) with the ANN toolbox of MATLAB software (MathWorks, Inc.; Natick, MA, USA). Multilayer feed-forward neural networks trained by an error back-propagation algorithm were conducted to evaluate the anti-glycated activities of polysaccharides of PG (PPG) and PL (PPL), respectively. Genetic algorithms were employed to optimize ultrasonic conditions for preparing PPG and PPL to obtain the maximum anti-glycated activities.

2. Experimental

2.1. Plant materials

Fresh, moderately mature *P. guajava* and *P. Littorale* fruits were purchased from a farm on Haiou Island, Guangzhou city, Guangdong province, China. Fruits free of defects were selected for uniform size and light-green color. These fruits were sliced and dried at 75 °C in an oven to a constant weight. Then, they were cut into small 2-g pieces.

2.2. Chemicals

Bovine serum albumin (BSA), aminoguanidine, sodium azide, glucose and DPPH• (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sulfuric acid, and phenol were purchased from Guangzhou Reagent Co. (Guangzhou, China). All the other reagents used in this study were of analytical grade.

2.3. Extraction and quantification of PPG and PPL

Fifteen grams of guava fruits were extracted with 250 mL distilled water under the selected ultrasonic conditions with an ultrasonic cleaner (KH-400KDB, Hechuang Ultrasonic Equipment Co., KunShan, China). The extract was filtered through filter paper and concentrated to 50 mL using a rotary evaporator at 60 °C under vacuum. Anhydrate ethanol was added until the ethanol concentration reached 75% as measured by an alcoholmeter. The mixture was maintained for 24 h at room temperature to precipitate polysaccharides, which were then lyophilized to yield the final precipitate powder used for further activity assays. Polysaccharide content was determined with a phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose as a standard.

2.4. Anti-glycated activity assay

Anti-glycated activities were determined with the method described by Zhao, Yang, Yang, Jiang, and Zhang (2007) with some modifications. A stock solution with 2% (w/v) BSA, 0.1 M glucose, and 0.1% (w/v) sodium azide in pH 7.4 phosphate buffer was prepared. Aminoguanidine was used as positive control. PPG or PPL were dissolved in the stock solution to a concentration of 0.5 mg/mL. The negative control was free of polysaccharides and aminoguanidine. Bacteria were removed by membrane filtration with a 0.22-μm pore-size filter. Solutions were store in a 10-mL centrifuge tube and incubated in the dark at 37 °C for 3 weeks. The content of advanced glycated end-products (AGEs) was determined with a fluorospectrophotometric method with an excitation wavelength of 370 nm and an emission wavelength of 440 nm. The percentage of anti-glycated activity was calculated as

$$G\% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

where A_{control} and A_{sample} represent the fluorescent determination of the negative control and sample group, respectively.

2.5. DPPH• free radical scavenging activity assay

The DPPH• free radical scavenging capacities of PPL and PPG were measured by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) test, according to the method of Yan, Kong, and Ou (2012). Briefly, a 1 mM solution of DPPH• in ethanol was prepared daily before ultraviolet (UV) measurements. Ascorbic acid was used as positive control. Next, 100 μL of various concentrations of crude polysaccharides or ascorbic acid in ultra-pure water was added to 100 μL freshly prepared DPPH•, and the mixture was shaken mildly. DPPH• free radical reduction was measured by absorbance at 517 nm after incubation for 30 min at room temperature. A_{max} was the value free of samples and adjusted to 200 μL with distilled water. A_{p} was the value with the samples, and A_{c} was 100 μL DPPH• free sample that was and adjusted to 200 μL with anhydrate alcohol. The inhibition percentage was calculated from the following equation: $S\% = 1 - \left(\frac{A_{\text{p}} - A_{\text{c}}}{A_{\text{max}}}\right)$

The free radical scavenging activity was expressed as EC_{50} values in mg/mL.

2.6. ANN modeling

The most common ANN architecture is based on feed-forward structure, which is often associated to the traditional back-propagation training algorithm used for computing ANN weights and biases. Back-propagation and probabilistic neural networks use supervised learning, whereas most self-organizing neural networks are based on unsupervised learning (Cimpoiu et al., 2011). In this work, according to the method of Yang, Zhao, and Jiang (2008), a feed-forward neural network trained with an error back-propagation algorithm was introduced using MATLAB (Version 7.0) Neural Network Toolbox to model the anti-glycated activity as a function of independent ultrasonic treatment variables. Multilayer feed-forward neural networking has shown to be an agreeable universal approximator of non-linear functions (Dreyfus & Dreyfus, 2003). Ultrasonic power, temperature, and time were chosen as the input parameters. A 3-N-1 neural network was conducted so that the neurons would be properly trained by the training set. Supervised learning was used to train this network by comparing the predicted and desired outputs. The weights of the network were adjusted by an error back-propagation algorithm. The weighted output was then activated using the hyperbolic tangent sigmoid transfer function. 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.