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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.

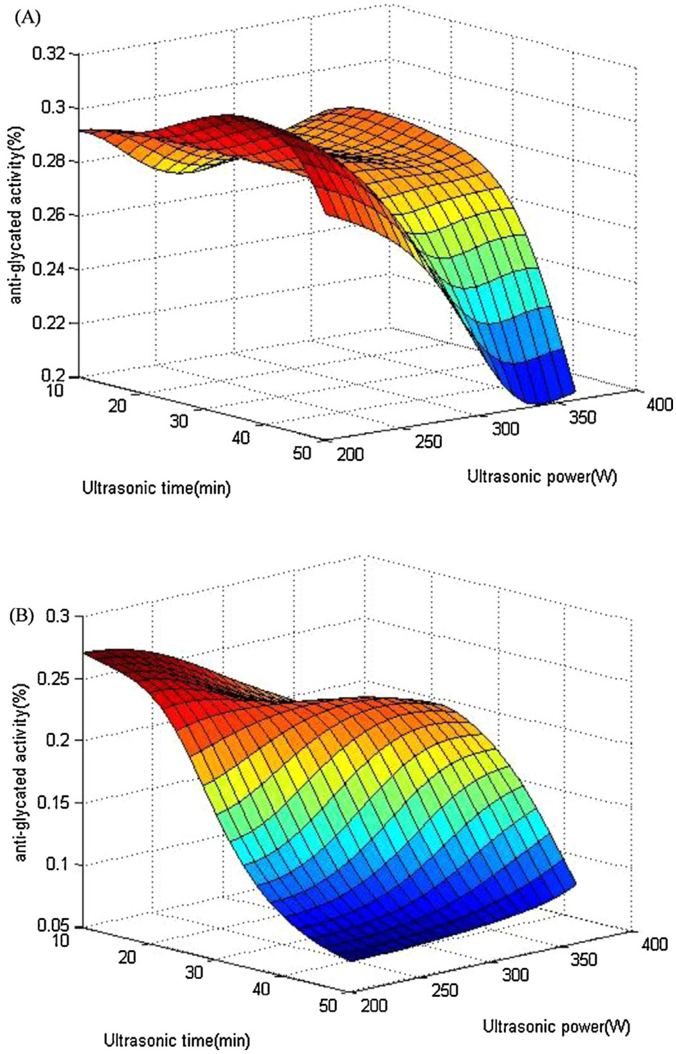

Fig. 1. Response surface plot showing the effects of ultrasonic power, time, and their interactions on the anti-glycated activities of PPL (A) and PPG (B). The ultrasonic temperature was 40 °C.

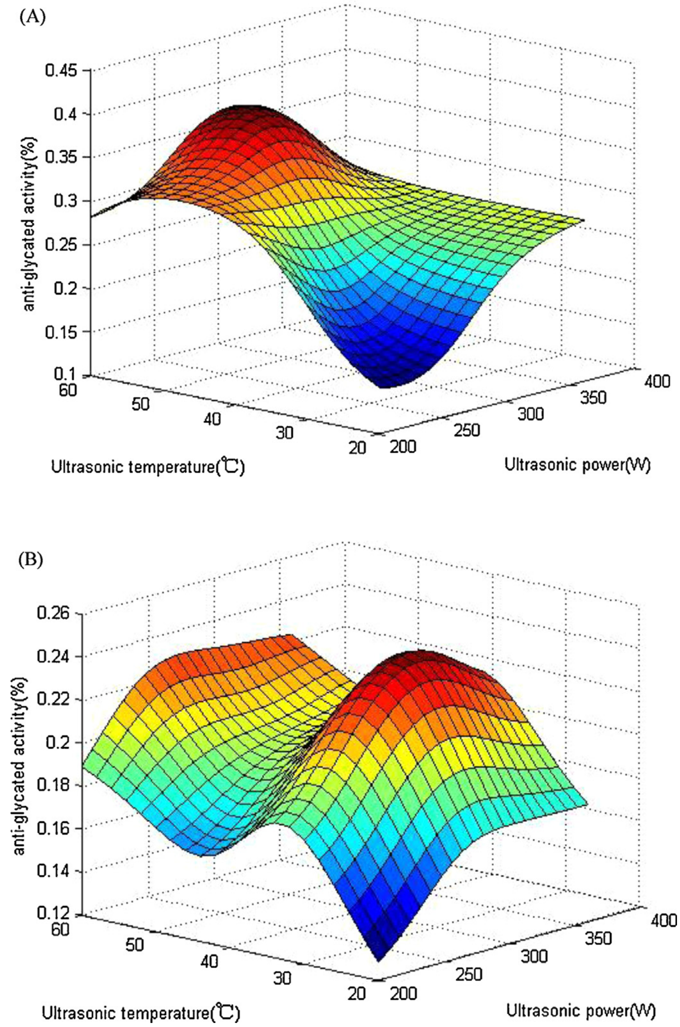

Fig. 2. Response surface plot showing the effects of ultrasonic power, temperature, and their interactions on the anti-glycated activities of PPL (A) and PPG (B). The ultrasonic time was 30 min.

2.7. Ultrasonic treatment condition optimization

Genetic algorithms were used to optimize ultrasonic conditions for the highest anti-glycated activity after the feed-forward neural network model was properly trained.

2.8. Statistical analysis

Data are expressed as means of triplicate determinations. Statistical calculations were conducted with Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) to determine correlations.

3. Results and discussion

3.1. Effects of ultrasonic power, temperature, and time

The effects of ultrasonic conditions on the anti-glycated activity of PPG and PPL are shown in Figs. 1–3. The activities of the two samples were horizontally compared, and the interaction between extracted conditions and bioactivities were revealed. As shown in Fig. 1(A) and (B), ultrasonic power of PPG and extracted time of PPL reduced anti-glycated activities when the extracted temperature was 40 °C, whereas ultrasonic time and ultrasonic power did affect the bioactivities of PPL and PPG, respectively. A similar positive tendency of ultrasonic power is seen in Fig. 2(A) and2(B) at a constant time of 30 min. Anti-glycated activity of PPL increased to a certain value with increased temperature and then began to decrease, while PPG activity increased again after initially decreasing. The effects of ultrasonic time and temperature were completely different for PPL and PPG when the ultrasonic power was constant at 280 W. PPL activity increased at higher temperature and decreased over time; PPG activity decreased with temperature, and time did not seem to have a noticeable effect (Fig. 3(A) and (B)). Most importantly we found that each factor exhibited a complicated correlation with anti-glycated activity.

3.2. Free-radical scavenging activity

The antioxidant activities of PPG and PPL were assessed by measuring their ability to scavenge DPPH$^{\bullet}$ radicals. PPG was obtained by ultrasonic conditions: 10 min, 200 W, and 37 °C, which optimized anti-glycated activity. The best conditions for PPL were 12 min, 256 W, and 60 °C. The two preparations were able to inhibit DPPH$^{\bullet}$ radical activity with EC_{50} values of 1.65 mg/mL for PPG and

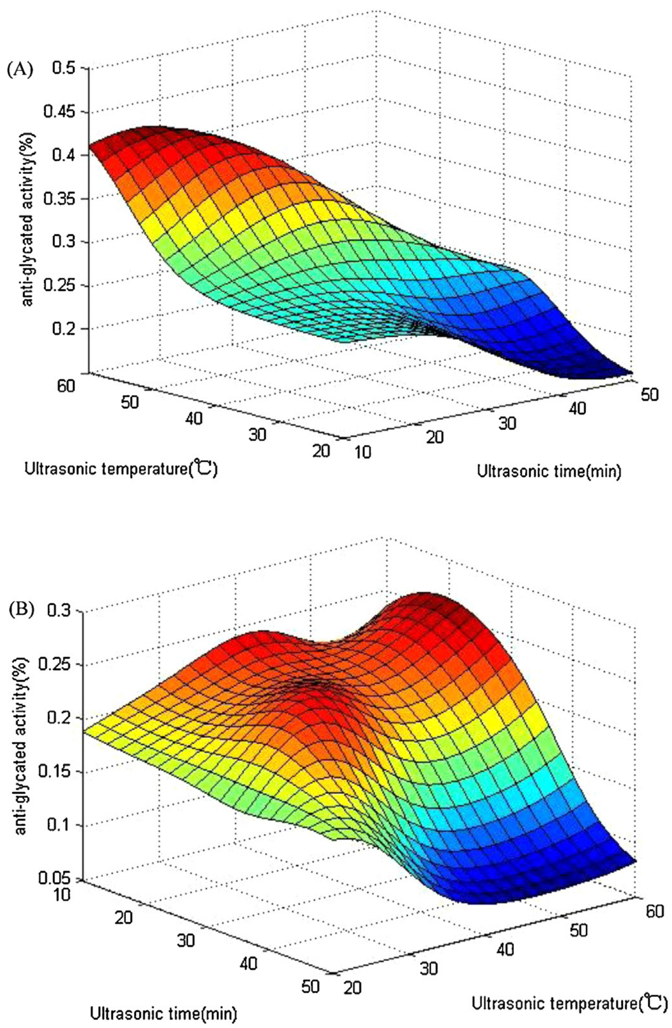

Fig. 3. Response surface plot showing the effects of ultrasonic time, temperature, and their interactions on the anti-glycated activities of PPL (A) and PPG (B). The ultrasonic power was 280 W.

1.43 mg/mL for PPL. The antioxidant activities were apparently dose dependent but lower than that of ascorbic acid (Fig. 4).

Glycation has been confirmed to have a significant role in normal aging (Yang et al., 2009) and the development of chronic diabetic complications, including cataract (Singh, Barden, Mori, & Beilin, 2001). Non-enzymatic glycation of proteins by reduction with carbohydrates and further oxidation, rearrangements, and eliminations would result in AGE formation (Wolff, Jiang, &

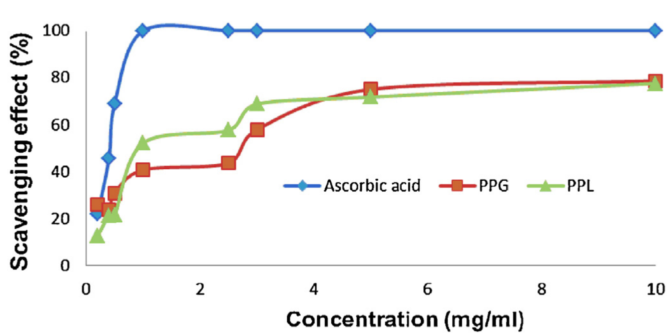

Fig. 4. Scavenging effects of PPL, PPG, and ascorbic acid on DPPH• free radicals. Each sample was assayed in triplicate at each concentration. Ultrasonic conditions for PPG were: 10 min, 200 W, and 37 °C, and those for PPL were 12 min, 256 W, and 60 °C.

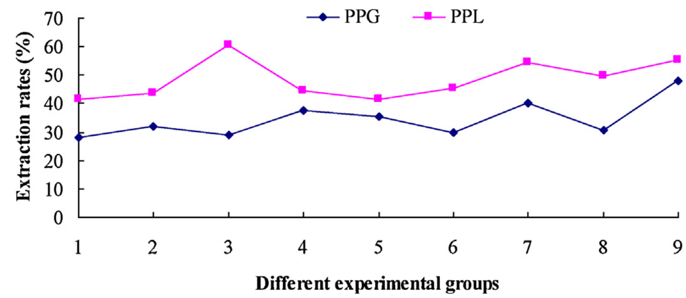

Fig. 5. The extraction rates of polysaccharides obtained at nine different extracted conditions.

Hunt, 1991), which might further alter peptide structure, function, and stability. There have been many reports of AGE accumulation under pathophysiological conditions, such as diabetes and aging (Ge, Zhang, & Sun, 2009). Therefore, identifying effective inhibitors of AGE formation will have a meaningful impact on human health. Peyroux and Sternberg (2006) have reported that trapping reactive dicarbonyl species and inhibiting their oxidation can prevent AGE formation. In this work, PPL and PPG were demonstrated to have DPPH•-scavenging and anti-glycated activities. It was hypothesized that the antioxidant activity of these polysaccharides should be one of the reasons for their anti-glycated activity. They can compete against glucose to react with proteins and decrease the effective protein concentration. This might be another pathway that contributes to inhibiting AGE formation (Yang et al., 2009). We found that PPG and PPL showed different anti-glycated activities depending on ultrasonic treatment conditions. It has been suggested that ultrasonic treatment can modify polysaccharide structure and molecular weight to influence its ability to scavenge DPPH• free radicals (Zhang, Zhang, Cheung, & Ooi, 2004). An alternative explanation is that different extraction conditions influence the extracted rates of active polysaccharides, which affects their bioactivities. The polysaccharide extraction rates for nine different conditions (listed in Table 1 as training set) are shown in Fig. 5.

3.3. *Evaluation of model predictability*

Statistical calculations were conducted to calculate correlations between predicted and actual values. Three ultrasonic factors used for in the incorporated network were ultrasonic time (10–50 min), power (200–360 W), and temperature (20–60 °C). The input matrix and anti-glycated activity values are shown in Table 1. According to the rule of neural networks, 13 input values were divided into 2 sets: 9 for the training set and 4 for the testing set. Fig. 6 indicates the plots of predicted and actual activity values of PPL and PPG, as well as R^2, an important index that reflects the fitness degree of the mathematical model (Nath & Chattopadhyay, 2007). The model fits well the actual data when R^2 approaches 1 (Sin, Yusof, Hamid, & Rahman, 2006). The R^2 values were 0.9781 for PPG and 0.9841 for PPL, which implied good fits between the values predicted by the network and the actual values.

Genetic algorithms were introduced to optimize ultrasonic extraction conditions because their most appreciated features include their prediction-generalization and classification abilities in the presence of noise, e.g., for complex systems with many interrelated parameters (Khanchi et al., 2007; Pazourek et al., 2005). The application of these algorithms showed that the optimal ultrasonic conditions to obtain maximum anti-glycated activity were 10 min, 200 W, and 37 °C for PPG and 12 min, 256 W, and 60 °C for PPL. The predicted activity values were 27.2% of PPG and 42.2% of PPL. The actual anti-glycated activities were 25.9% of PPG and 41.9% of PPL under the respective optimal conditions, which were very close to predicted values. These results indicate that the ANN showed good

Table 1
The predicted neural network values and actual anti-glycated activity values.

Experiment	Ultrasonic time (min)	Ultrasonic power (W)	Ultrasonic temperature (°C)	Anti-glycated activity of PPL (%)		Anti-glycated activity of PPG (%)	
				Predicted	Actual	Predicted	Actual
Training set							
1	10	200	20	20.5	21.4	19.8	19.8
2	10	280	40	26.9	26.4	24.2	24.2
3	10	360	60	29.2	28.1	23.1	23.1
4	30	200	40	30.8	31.2	22.9	16.7
5	30	280	60	34.9	33.8	22.9	22.9
6	30	360	20	28.4	28.4	23.9	23.9
7	50	200	60	21.1	24.5	9.31	9.31
8	50	280	20	15.8	15.2	16.7	17.5
9	50	360	40	20.3	20.4	10.03	10.0
Testing set							
1	20	240	30	22.4	18.9	21.4	21.9
2	20	320	50	31.8	30.6	20.5	20.8
3	40	240	50	27.9	27.5	9.37	14.3
4	40	320	30	24.3	23.8	21.3	20.1

The polysaccharide extraction rates for nine different conditions of training set and four different conditions of testing set.

prediction and optimization capabilities. At the same time, PPL had better bioactivities than PPG in both assays.

ANNs are rapidly being adapted for use in chemistry (Li, Chen, Xiang, & An, 2000) but only a few applications for food and natural product chemistry have been reported so far. Experimental results show that ANNs are reliable, fast, and cost-effective tools for predicting polysaccharide bioactivity. Therefore, ANNs can be used to model nutraceutical properties or predict food ingredient quality.

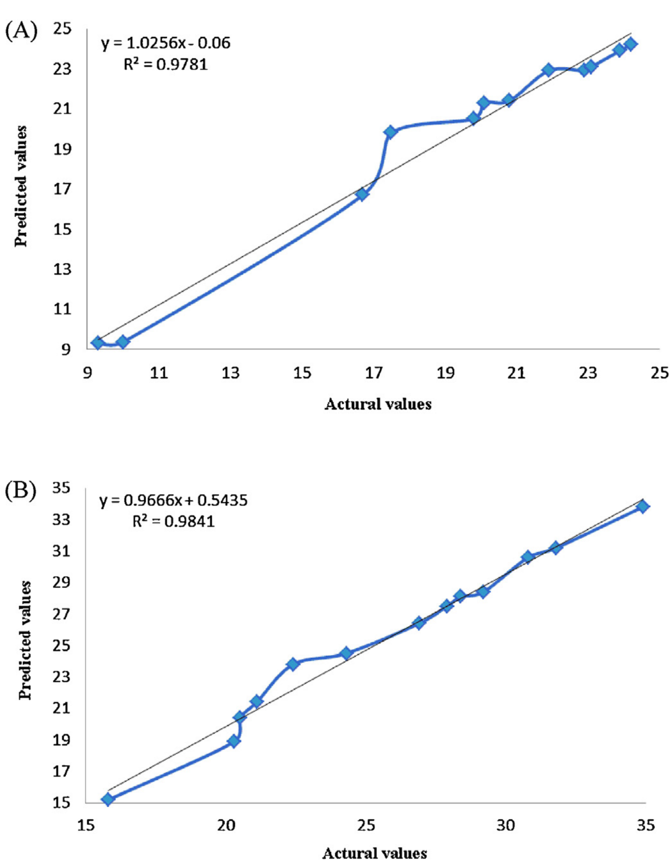

Fig. 6. Correlation between the predicted and actual values of anti-glycated activity (A for PPG and B for PPL).

4. Conclusions

In this study, PPG and PPL were extracted under different ultrasonic conditions. Models of anti-glycated activity were generated with the ANN toolbox of MATLAB, and response surface plots indicated that ultrasonic power, time, and temperature had complex and significant effects on the anti-glycated activity of both polysaccharides. The R^2 of PPG and PPL were determined to be 0.9841 and 0.9781, respectively, which suggested a good fit and generalization of the ANN. The highest anti-glycated activity of PPG was predicted to be 27.2% for ultrasonic conditions of 10 min, 200 W, and 37 °C, while the predicted anti-glycated activity of PPL was 42.2% for 12 min, 256 W and 60 °C. EC_{50} values for the DPPH$^•$ assay were 1.65 mg/mL for PPG and 1.43 mg/mL for PPL by the optimal ultrasonic-extract conditions. The data show that both PPG and PPL possess anti-glycated and antioxidant activities, but PPL has stronger effects. Importantly, the experimental anti-glycated activities under the respective optimal conditions were very close to the predicted values. As a result, probabilistic ANN may be used as a trustworthy tool for validation and genuineness assessment of food ingredients. We are currently researching the relationship between polysaccharide structure and anti-glycated activity in *P. guajava* and *P. littorale*.

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