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Optimization of ultrasonic-assisted extraction of phenolic compounds from guava seed and its antioxidant activity

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Abstract

In order to improve development and utilization value of guava, the extraction of phenolic compounds from guava seed and its antioxidant activity were evaluated. Based on the single factor experiment, an optimization of 3-factor and 3-level by response surface methodology was designed and carried out to construct regression model and obtain optimal condition of ultrasonic power, ethanol concentration and water to raw material ratio. Then capacities of scavenging hydroxyl free radical and superoxide anion free radical were used to evaluate antioxidant capacity of phenolic compounds extraction. Results showed that the optimal technological condition was determined as ultrasonic power of 345 W, ethanol concentration of 70%, and water to raw material ratio of 22 mL/g. Under these conditions, the experimental yield of phenolic compounds was 2.302 mg/g, which agreed closely with the predicted value (2.340 mg/g). At the same time the study of antioxidant activity exhibited stronger antioxidant activity of phenolic compounds in guava seed than ascorbic acid. Its maximal scavenging rates for hydroxyl free radical and superoxide anion free radical were 62.38% and 80.23%, and the values of IC_{50} were 154 μg/mL and 552 μg/mL, respectively. Hence the phenolic compounds in guava seed is abundant and also showed stronger antioxidant activity, and ultrasonic-assisted extraction could get high extraction rate of phenolic compounds.

Keywords: Phenolic compounds, Guava seed, Ultrasonic-assisted extraction, Antioxidant activity.

1. Introduction

Guava is a green plant in *Psidium* L, *Myrtaceae*, containing sugar, fat and varieties of functional ingredient such as vitamin B, vitamin C, organic acid and so on [1, 2]. Guava fruit is delicious and valuable with much medicine value and health care [3, 4]. Especially that guava is rich in vitamin C, polyphenol, and other antioxidant regents which have strong activity to clear free radical in vitro away, hence who often eats guava will promotes metabolism, prolongs body age and prevents cancer of body [5, 6, 7]. Plant polyphenols were a group of phenol compounds with many hydroxyls and in essence the production in complicated secondary metabolism process of plant body, many part of plants can be traced the trail of phenolic compounds such as peel, leaf and fruit [8]. Phenolic compounds are extracted using conventional water extraction and organic solution extraction [9]. Many methods are less used alone because of their low extraction rate, whose assistance extractions in turn obtain more and more application chance in the study of extracting phenolic compounds technology coupling with microwave or ultrasonic which noticeably raise extraction rate [10]. In fact many reports and studies have extracted phenolic compounds out from many plants [11, 12]. However, extraction of phenolic compounds in guava seed is less studied, different extraction methods and conditions are evaluated to understand their influence on the phenolic compounds extraction. Meanwhile guava seed is rich in phenolic compounds. Based on the single factor experiment, the extraction technology of phenolic compounds from guava seed was studied on the optimal condition of main extraction factors by response surface methodology, at the same time phenolic compounds of guava seed was tested and analyzed for its antioxidant activity to scavenge hydroxyl free radical and superoxide anion free radical.

2. Materials and methods

2.1 Single factor experiment of phenolic compounds extraction

Fresh guava as the raw material was used in the experiment. Guava seed taken from guava was dried in electro-thermostatic blast oven (DGG-9140A, Shanghai Qixin Science Instrument Co., LTD, China) to constant weight and then broken into powder for using. In each experiment group, 2.5 g guava seed powder was experiment sample, extraction solution was ethanol solution of different concentration and ultrasonic cell disruption system (XO-1200, Nanjing Xianou Instrument Making Co., LTD, China) was used to provide ultrasonic environment. Single factor experiment was carried out to study the influence of extraction technology factors including ultrasonic power, ethanol concentration and water to raw material ratio on the extraction of phenolic compounds in guava seed and determine their preliminary range for superior extraction effect. Ultrasonic power was studied in the 5 levels namely 200 W, 250 W, 300 W, 350 W and 400 W; water to raw material ratio was studied in the 5 levels namely 5 mL/g, 10 mL/g, 15 mL/g, 20 mL/g and 25 mL/g; ethanol concentration was studied in the 5 levels namely 40%, 50%, 60%, 70% and 80%.

2.2 Optimization of ultrasonic-assisted extraction by response surface methodology

The response surface method (RSM) consists of a group of mathematical and statistical procedures that can be used to study the relationship between one or more responses and a number of independent variables. In addition to analyzing the effect of independent variables, the experimental methodology generates a mathematical model that accurately describes the overall process. In the experiment a Box-Behnken design of 3-

factor and 3-level was used to optimize response factors including ultrasonic power, ethanol concentration and water to raw material ratio with extraction rate (mg/g) as response value according to single factor experiment. The levels and factors in the response surface experiment were as follows (Table 1).

Table 1: Design of extraction optimization of phenolic compounds in guava seeds

Level	Factor		
	X_1: Ultrasonic power/W	X_2: Ethanol concentration/%	X_3: Water to raw material ratio/(mL/g)
-1	300	60	15
0	350	70	20
1	400	80	25

2.3 Activity analysis of scavenging hydroxyl free radical

Its capacity to scavenge hydroxyl free radical was testing using Fenton reaction compared with that of ascorbic acid in similar operation. 6 colorimetric cylinders of 10 mL were prepared and these kinds of reaction solution were added into the colorimetric cylinders in order including 9.0 mmol/L $FeSO_4$ solution taken 1 mL, 9.0 mmol/L ethanol-salicylic acid solution taken 1.00 mL and 8.8 mmol/L H_2O_2 solution taken 1.00 mL. Then following was to add phenolic compounds extraction liquid of guava seed of different volume from 0 mL to 5.00 mL into the colorimetric cylinders and finally add hyperpure water to 10 mL. Eventually the colorimetric cylinders got through water bath at 37 °C lasting 15 min. Their absorbance values were tested in 510 nm and scavenging rate was obtained according to the following equation (1). In the equation, A_0 stood for the absorbance of the colorimetric cylinder without phenolic compounds extraction liquid of guava seed, A_x stood for the absorbance of the colorimetric cylinder with phenolic compounds extraction liquid of guava seed of different volume and A_{x0} stood for the absorbance of the colorimetric cylinder with phenolic compounds extraction liquid of guava seed of different volume and no H_2O_2 solution.

$$Scavenging \quad rate/\% = \frac{A_0 - (A_x - A_{x0})}{A_0} \times 100\% \quad (1)$$

2.4 Activity analysis of scavenging superoxide anion free radical

Its capacity to scavenge superoxide anion free radical was testing using ortho benzene three phenol colorimetry compared with that of Vc in similar operation. 6 colorimetric cylinders of 10 mL were prepared and these kinds of reaction solution were added into the colorimetric cylinders in order including 0.05 mmol/L Tris-HCl buffer solution (pH8.2) taken 4.50 mL and 0.05 mol/L EDTA taken 1.00 mL. And following was to add phenolic compounds extraction liquid of guava seed of different volume from 0 mL to 5.00 mL and take 10 min for water bath at 25 °C. Then 9 mmol/L ortho benzene three phenol solution was added 0.1 mL in and reaction time should be kept in 60 min. Eventually 12 mol/L HCl solution was added 0.05 mL in and hyperpure water was add to 10 mL. Their absorbance values were tested in 325 nm and scavenging rate was obtained according to the following equation (2).

$$Scavenging \quad rate/\% = \frac{Ac - As}{Ac} \times 100\% \quad (2)$$

In the equation, A_c stood for the absorbance of the colorimetric cylinder without phenolic compounds extraction liquid of guava seed, As stood for the absorbance of the colorimetric cylinder with phenolic compounds extraction liquid of guava seed of different volume.

2.5 Determination of phenolic compounds concentration

Phenolic compounds concentration in polyphenol extraction liquid of guava seed was determined in Folin-ciocalteu method coupling with standard curve. Gallic acid was used as standard sample and a visible light spectrometer (722N, Shanghai Yidian Scientific Instrument Co, LTD, China) was used for the testing of absorbance. Firstly, 0.05 mg/mL gallic acid standard solution was prepared, then gallic acid solution of different volume from 0.0 mL to 8.0 mL was placed in 50 mL brown volumetric flasks. Then following was to add 4.0 mL Folin-Ciocalteu regent and 10% Na_2CO_3 solution taken 4.0 mL. The whole was diluted to 50 mL and stored in 1 h in room temperature and dark environment. Their absorbance values were tested in 760 nm and each was tested 3 times.

3. Results & Discussion

3.1 Standard curve of gallic acid

The absorbance values of gallic acid were employed to make a standard curve with the concentration of gallic acid and the absorbance.The regression equation of standard curve was as follows: y=0.1126x+0.0104, R^2=0.9987, and the curve nearly exhibited a straight line in the range of 0-8.0 μg/ml. The standard curve could be used to ensure extraction rate of phenolic compounds in guava seed.

3.2 Effect of ultrasonic power on phenolic compounds extraction

Ultrasonic power works in the exposure of polyohenol to release medium where liquid penetrated into the dried powder, dissolved the phenolic compounds and subsequently diffused out from the powder. While other extraction parameters were as follows namely water to raw material ratio of 10 mL/g and ethanol concentration of 60%, the extraction rate of phenolic compounds was shown in Fig.1 with the change of ultrasonic power. Different ultrasonic power was shown in Fig.1 to have different extraction rates of phenolic compounds. Extraction rate rose influenced by increasing ultrasonic power and the influence of ultrasonic power in 200~250 W was bigger than that of ultrasonic power in 250~350 W, which may be explained by the break of cell wall. In a word ultrasonic power of 350 W make extraction rates of 1.23 mg/g the highest, ultrasonic power of 400 W would in turn decrease extraction rate. The reason may be that ultrasonic will break chemical structure of phenolic compounds. Therefore, ultrasonic power of 350 W was considered to be the optimal ultrasonic power in present experiment.

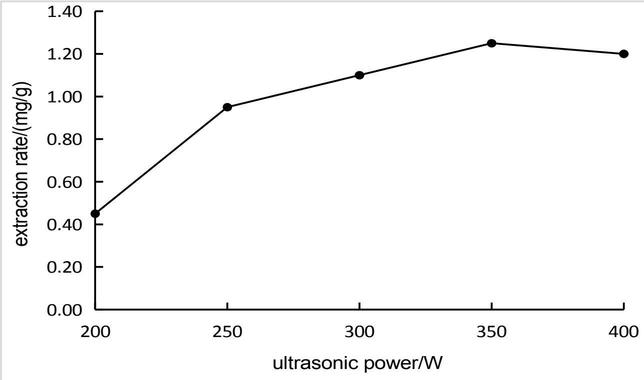

Fig 1: Effect of ultrasonic power on extraction rate

3.3 Effect of water to raw material ratio on phenolic compounds extraction

The effects of water to raw material ratio were shown in Fig.2 on extraction rate of phenolic compounds. Extraction was carried out in different water to raw material ratio while other extraction parameters were as follows: ethanol concentration of 60% and ultrasonic power of 300 W. It was shown in Fig.2 that water to raw material ratio had great impact on extraction rate of phenolic compounds in guava seed. Extraction rate rose influenced by the increasing of water to raw material ratio firstly, extraction rate reached the peak of 1.65 mg/g when water to raw material ratio was 20 mL/g, then the extraction rate started to decrease when water to raw material ratio was 25 mL/g with the extraction rate of 1.25 mg/g. In a word extraction rate showed the current to increase first raised by increasing water to raw material ratio and then decrease dropped by excessively high water to raw material ratio. The reason may be that more solution has bigger extraction capacity and contributes to the extraction of phenolic compounds, however, overmuch solution decreases phenolic compounds concentration, increases the difficulty of recollection and also leads to severe waste. Therefore, the water to raw material ratio of 20 mL/g was considered to be the best in present experiment.

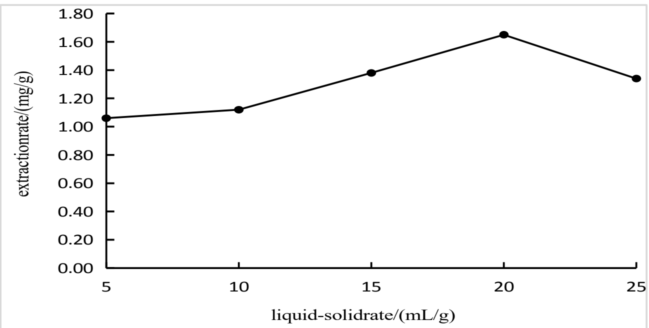

Fig 2: Effect of water to raw material ratio on extraction rate

3.4 Effect of ethanol concentration on phenolic compounds extraction

The effects of ethanol concentration were shown in Fig.3 on extraction rate of phenolic compounds. Extraction was carried out in different ethanol concentration conditions while other extraction parameters were as follows: water to raw material ratio of 10 mL/g and ultrasonic power of 300 W. it showed in Fig.3 that the extraction rate reached the peak of 2.12% as ethanol concentration was 70%. Extraction rate rose influenced by the increasing of ethanol concentration firstly, then the extraction rate started to decrease when ethanol concentration was 80% with the extraction rate of 1.55 mg/g. In a word extraction rate showed the current to increase first raised by increasing ethanol concentration and then decrease dropped by excessively high ethanol concentration. The reason may be that more ethanol concentration contributes to break cell wall and chemical bond such as H-bond between phenolic compounds and other compounds; in 70% ethanol environment phenolic compounds polarity was close to extraction liquid polarity and phenolic compounds dissolution was the best. But excessively high ethanol concentration leads to decrease the polarity of solution and increase the volatility. Therefore, ethanol concentration of 70% was the optimal ethanol concentration in present experiment.

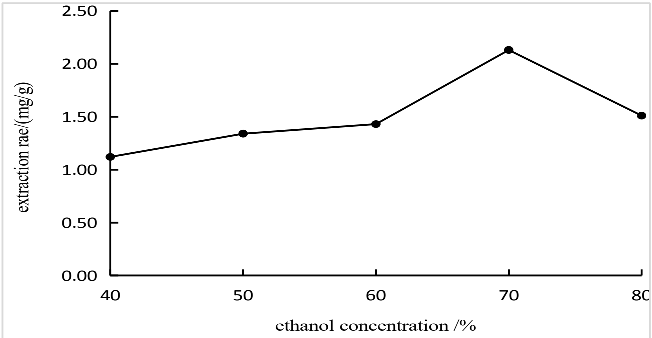

Fig 3: Effect of ethanol concentration on extraction rate

3.5 Technology optimization by response surface methodology

Taking single factor experiment into consideration, a Box-Behnken experiment was carried out to obtain the optimal extraction rate (mg/g) with ultrasonic power, ethanol concentration and water to raw material ratio as response factors. Experimental results and variance analysis were showed in Table 2.

Table 2: The results of extraction optimization of phenolic compounds in guava seed

No.	X1	X2	X3	Extraction rate（mg/g）
1	-1	-1	0	1.902
2	1	-1	0	1.526
3	-1	1	0	1.548
4	1	1	0	1.734
5	-1	0	-1	1.494
6	1	0	-1	1.605
7	-1	0	1	2.048
8	1	0	1	1.858
9	0	-1	-1	1.492
10	0	1	-1	1.394
11	0	-1	1	1.809
12	0	1	1	1.984
13	0	0	0	2.301
14	0	0	0	2.342
15	0	0	0	2.245

Experimental results were used to construct the regression model of extraction rate of phenolic compounds including 3 response factors namely ultrasonic power, ethanol concentration and water to raw material ratio in the software platform of Design-Expert 8.0. The equation was shown as follows.

$$Y=-26.30325+0.060857\times X_1+0.36349\times X_2+0.49465\times X_3+0.000281\times X_1X_2-0.00301\times X_1X_3+0.001365\times X_2X_3-0.0001074\times X_1^2-0.0035\times X_2^2-0.01105\times X_3^2$$

P value of model turned out highly significant of the regression model ($P<0.0001$). Residual error value proved random error negligible. The regression model could explain 99.00% of change of response values ($R^2=0.9900$, $R^2_{adj}=0.9721$). More importantly, lack of fit was non-significant in regression model with its P value equal to 0.4698.

3.6 Interaction analysis of technology parameters

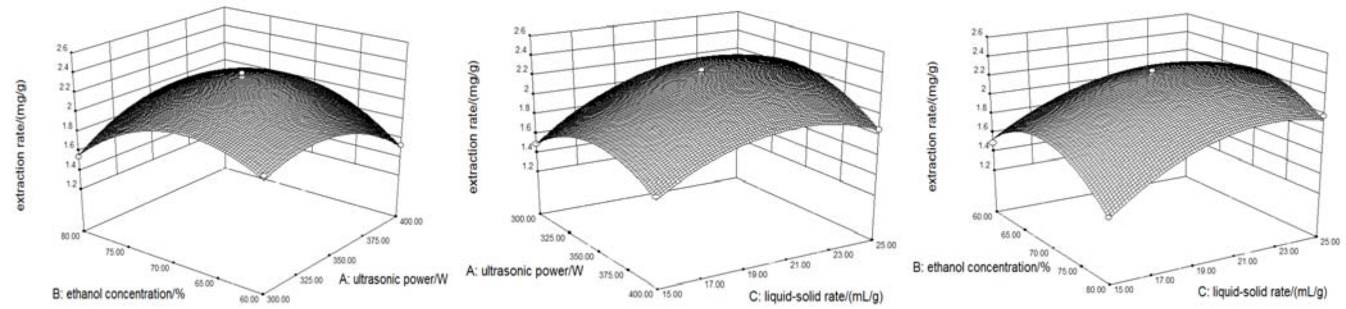

Fig 4: Response surface plots of interaction on extraction rate of phenolic compounds

In Fig.4 interaction of ultrasonic power, ethanol concentration and water to raw material ratio on extraction rate of phenolic compounds were shown. The increasing of ultrasonic power made the extraction rate rise firstly and then decrease, and the change of ethanol concentration strongly affected the effect tendency of ultrasonic power on the extraction rate; the increasing of ethanol concentration made the extraction rate rise firstly and then decrease, and the effect of ethanol concentration was strongly affected with ultrasonic power changing. Thus, the interaction of ultrasonic power and water to raw material ratio was significant, which was corresponding to the significant interaction of X_1X_3. The extraction rate rose firstly and then decreased when increasing ethanol concentration, and the change of water to raw material ratio strongly affected the effect tendency of ethanol concentration on the extraction rate, the increasing of water to raw material ratio made the extraction rate rise firstly and then decrease, and the effect of water to raw material ratio was strongly affected with ethanol concentration changing. Thus, the interaction of ethanol concentration and water to raw material ratio was significant, which was corresponding to the significant interaction of X_2X_3.

3.7 Obtaining the optimal technology and verification experiment

Ultrasonic assisted extraction technology of phenolic compounds in guava seed was optimized using Design Expert 8.0 to process and analyze response surface experimental results. In the optimal technology ultrasonic power was 344.73 W, ethanol concentration was 70.12% and water to raw material ratio was 21.97 mL/g. In 3 verification experiments, ultrasonic power was adjusted as 345 W, ethanol concentration was adjusted as 70% and water to raw material ratio was adjusted as 22 mL/g. Final result of 3 verification experiments was mean extraction rate of 2.302 mg/g, which had low relative error with predicted extraction rate of 2.340 mg/g. Hence, the regression model was proved again to be highly reliable, which could afford the model and prediction of ultrasonic assisted extraction technology of phenolic compounds in guava seed.

3.8 Activity analysis of scavenging hydroxyl free radical of phenolic compounds

Phenolic compounds extraction liquid of guava seed owned the capacity shown in Fig.5 to scavenge hydroxyl free radical. It showed in Fig.5 that the activity of scavenging hydroxyl free radical showed increasing current influenced by increasing phenolic compounds concentration in phenolic compounds extraction liquid of guava seed and they exhibited a liner relationship in concentration range of 50µg/mL~250µg/mL ($R^2=0.9615$). The phenolic compounds extraction liquid of guava seed was also shown to have maximal scavenging rate of 62.38% and IC_{50} of 154 µg/mL for hydroxyl free radical, which was superior than Vc in scavenging hydroxyl free radical. Hence phenolic compounds extraction liquid of guava seed had stronger activity of scavenging hydroxyl free radical.

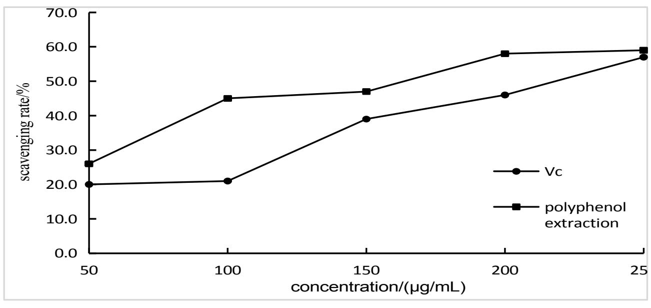

Fig 5: The hydroxyl radical scavenging activity of phenolic compounds in guava seeds

Conclusions

The ultrasonic-assisted extraction of phenolic compounds from guava seed was studied based on the foundation of single factor experiment to obtain the optimal condition. The optimal parameters were determined with maximal extraction rate of 2.340 mg/g where ultrasonic power 345 W, ethanol concentration 70% and water to raw material ratio 22.0 mL/g. Phenolic compounds extraction liquid of guava seed had stronger antioxidant activity with maximal scavenging rate of 62.38% and IC_{50} of 154 μg/mL for hydroxyl free radical and maximal scavenging rate of 80.23% and IC_{50} of 552 μg/mL for superoxide anion free radical. Hence phenolic compounds as a antioxidant compound in guava seed could play important role in health care, for example, preventing cancer because of free radical out of balance and body sharp aging, guava seed and its phenolic compounds extraction liquid had great application potential and the study of ultrasonic assisted extraction of phenolic compounds in guava seed was indispensable for the reason above.

Acknowledgments

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