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Study on the extraction, antioxidant activity of polysaccharides from *Piteguo* fruit

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Ultrasonic-assisted extraction (UAE) of polysaccharides from *Piteguo* fruit (*Pyrus sinkiangensis*) was studied. The four parameters, i.e., ratio of water to raw material, extraction time, extraction temperature, and ultrasonic power were optimized by the Box-Behnken design (BBD). The structure of polysaccharide from Piteguo (PTGP) was analyzed preliminarily by infrared spectrum. Antioxidant activities of PTGP were detected in vitro. The results indicated that the extraction ratio of crude PTGP was up to 5.16% ± 1.81% under the optimized extraction conditions as follows: ratio of water to raw material 13:1 mL/g, extraction time 66 min, extraction temperature 70 °C and ultrasonic power 230 W, which is was well matched with the value (5.25%) predicted by the BBD model. Spectroscopic studies illustrated PTGP was composed of Glc with β-type pyranoid type sugar ring. Moreover, the results of antioxidant activity assay indicated that PTGP has antioxidant effect in a certain extent, and could be used as a potential natural antioxidant.

Keywords: Piteguo fruit; Polysaccharide; Ultrasonic-assisted extraction; Response surface methodology; Antioxidative activity

INTRODUCTION

Polysaccharides are polymeric carbohydrate molecules of long chain monosaccharide units, and are of plant and algae origin with the exception of few microbial derived exopolysaccharides [1]. Polysaccharides have important health care functions such as antitumor activity [2], radioprotection activity [3], antioxidant activity [4], *et al.*. As well as, Federico and others [5] found that exopolysaccharides play an indispensable role in enhancing hydraulic conductivity of biological soil crusts.

Piteguo fruit (PTG), which is originally known as Pinangguo fruit (Skins fruit), is classified as Pyrus sinkian-gensis, subfamily Maloideae, family Rosaceae. According to expert research, it proves to have strong plant vigor and environmental adaptation, for it can grow well in poor soil, and resist common diseases and insects. This kind of indigenous fruit contains several kinds of essential amino acids, sugars, crude fiber, vitamins (e.g. C, B1, B2), tannic acid for human beings and essential elements such as potassium, calcium, iron, and *etc*. It is also reported in traditional Chinese medicine theory that *Piteguo* fruit has important health care functions such as nourishing the stomach, moistening the lung, quenching thirsty, and etc. So it is considered as a novel green food with high nutritional value [6].

In our study, we applied ultrasonic-assisted extraction procedure to extract crude polysaccharides from *Piteguo* fruit. Box-Behnken design (BBD) [7], one type of RSM was introduced to optimize ultrasonic-assisted extraction technology condition of crude polysaccharides from *Piteguo* fruit and systematically analyze the effects of extraction parameters on their yields and their interactions. In addition, the structures and activities of polysaccharide were analyzed in order to provide the theoretical basis for application of polysaccharide from *Piteguo* fruit and full development of this kind of undeveloped fruit resource.

MATERIALS AND METHODS

Materials

Piteguo fruit was picked in Hezheng County, Linxia Hui Minority Autonomous Prefecture, Gansu Province in November, 2011. Before eating or application in this study, *Piteguo* fruit has to be treated properly to remove acerbity and become post-mature. Those soluble tannins distributed in the special tannin cells can be transformed into insoluble tannins by CO_2, alcohol and acetaldehyde produced from anaerobic fermentation in sealed containers. After post-maturation, the color of the fruit turns brown and the pulp feels much softer than the unripe one. Then, the treated (post-mature) fruits were transferred and stored at -20 °C for the following extraction experiments.

Methods

Extraction of crude polysaccharides from Piteguo fruit with ultrasonic-assisted treatment The treated *Piteguo* fruits were washed by distilled water

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and the peels were removed before being cut into small pieces. The process of crude polysaccharides extraction from *Piteguo* fruits by ultrasonic-assisted treatment was performed in an ultrasonic processor (KQ-250DB, Kunshan Ultrasonic Instruments Co., Ltd, Jiangsu, China). Then, impurity proteins, vitamins, lipids and other pulp components were separated by centrifugation in a 50 mL centrifuge tube [8]. The supernatant was withdrawn, concentrated in a vacuum concentrator and precipitated with 80% ethanol, and dried to get the crude PTGP by vacuum freeze drying (FD-1A-50, Boyikang Instruments Co., Ltd, Beijing, China).

Determination of the yield of polysaccharides from Piteguo fruit and extraction ratio The content of polysaccharide extracted from Piteguo fruit was measured by phenol-sulfuric acid method[9]. The freeze-dried polysaccharide sample was dissolved into distilled water, and optical density of this solution at 485 nm (OD_{485}) was measured. The content of polysaccharide in this sample was calculated according to equation of linear regression (Y=0.0607X-0.0569, R^2=0.9977) based on the standard curve whose horizontal coordinate and vertical coordinate denoted the concentration of glucose (μg/mL) and OD_{485}, respectively. The polysaccharide yield could be calculated as described [9].

Experimental design and statistical analyses In order to investigate the critical influencing factors in ultrasonic-assisted extraction, single-factor-test was employed in this experiment to determine the preliminary range of the variables including X_1 (ratio of water to raw material), X_2 (extraction time), X_3 (extraction temperature) and X_4 (ultrasonic power). Then, a three-level-four-factor BBD was adopted in the optimization study for the yields of *Piteguo* fruit water-soluble crude polysaccharides. Table 1 represents the coded and non-coded values of the experimental variables and 21 experimental points. Five replicates (17-21) were used to evaluate the pure error. Experimental data shown that response variables were fitted to a quadratic polynomial model.

FT-IR spectroscopy Infrared spectrum samples were prepared by potassium bromide tabletting. The dried PTGP power and potassium bromide powder were thoroughly ground. The mixed powder was made into a transparent sheet with about 1 mm thickness by a pneumatic tabletting machine. Then the samples were analyzed by a Fourier transform infrared spectrometer (Thermo Scientific Nicolet iN10) at 400 - 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Determination of antioxidant activities in vitro of PTGP

Hydroxyl radical scavenging assay The 10 mL of reaction mixture contained 2 mL of 200 mM sodium phosphate buffer (pH 7.4), 1.5 mL of 5.0 mM 1, 10-phenanthroline aqueous solution, 1.0 mL of 7.5 mM $FeSO_4$ aqueous solution, 1 mL of 0.1% H_2O_2 aqueous solution, 0.1 mL of the PTGP aqueous solution with different concentration (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0mg/mL, respectively) and distilled water. After incubating at 37 °C for 1 h, the absorbance of the mixture was measured at 510 nm. The scavenging activity of hydroxyl radicals production was calculated as described by Jin et al. [10].

Superoxide radical scavenging assay The sample was diluted to a different concentration (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/mL) with 50 mM Tris-HCl buffer (pH 8.2). Secondly, 5.0 mL of 50 mM Tris-HCl buffer (pH 8.2) and 0.1 mL of samples were incubated at 25 °C for 20 min, then 0.2 mL of 3 mM pyrogallol at the same temperature were added to the mixture and the reaction was kept at 25 ℃for 4 min, and the rate of absorbance change (A/min) of the solution was measured at 325 nm, with 50 mM phosphate buffer (pH 7.2) instead of sample as the control. The scavenging activity of superoxide radical was calculated according to by the Yuan and Gao [11].

Table 1. Independent variables and their levels used in the response surface design

Variables	Levels		
	-1	0	1
Extraction temperature (X_1) (°C)	65	70	75
Extraction time (X_2) (min)	50	60	70
Ratio of water to raw material (X_3) (mL/g)	10:1	15:1	20:1
Ultrasonic power (X_4) (W)	150	200	250

Effect of scavenging of (DPPH) radicals The solution of 0.2 mM DPPH in 60% ethanol was prepared before UV measurements. Then, 3.0 mL of the samples (0.1, 0.15, 0.20, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/mL) was added to 1.0 mL DPPH, and kept at room temperature for 30 min in the dark, and the absorbance at 525 nm was then measured against a blank. Ascorbic acid was used as positive controls. The scavenging activity of DPPH radical (%) was calculated according to the Shimada and others [12].

Determination of reducing power The reducing power of PTGP was evaluated according to Oyaizu [13] with necessary modifications. The reaction mixtures contained 2.5 mL phosphate buffer (pH 6.6, 0.2M), 2.5 mL potassium ferricyanide (1%, w/v) and PTGP (0.1, 0.15, 0.20, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/mL). After incubating at 50 ℃for 20 min, 2.5 mL of trichloroacetic acid (10%, w/v) was added into the mixture for terminating the reaction, and then centrifuged at 1500×g for 10 min. An aliquot of 2.5 mL supernatant was collected and mixed with 2.5 mL deionized water and 0.5 mL $FeCl_3$ (0.1%, w/v). After incubating at room temperature for 15 min, the absorbance at 700 nm of the mixture was measured, using ascorbic acid as a positive control. Higher absorbance of the reaction mixture indicated higher scavenging activity of free radicals.

Statistical analysis All the data were exhibited as mean values of three replicate determinations. Difference was considered to be significant when $p<0.05$. Statistical analysis involved use of the Origin Pro software package 8.5 (Origin Lab Corp.) and Design Expert software Version 8.0.5 (Stat-Ease Inc.).

RESULTS AND DISCUSSION

Effect of extraction temperature on extraction yield of polysaccharides In this experiment, the effects of different extraction temperature on the yield of PTPG were studied, while the other extraction conditions such as the ratio of water to raw material, extraction time, ultrasonic power were fixed at 15 mL, 60 min, 150 W respectively. As shown in Figure 1A, the extraction yield of polysaccharides continued to increase with the increasing of extraction temperature and reached at the peak value (5.76%) when extraction temperature was 70 °C. However, the yield was noticed to decrease when the extraction temperature exceeded 70 °C, which may be caused by the degradation of polysaccharides. Thus, the optimum extraction temperature was 70 °C according to the results.

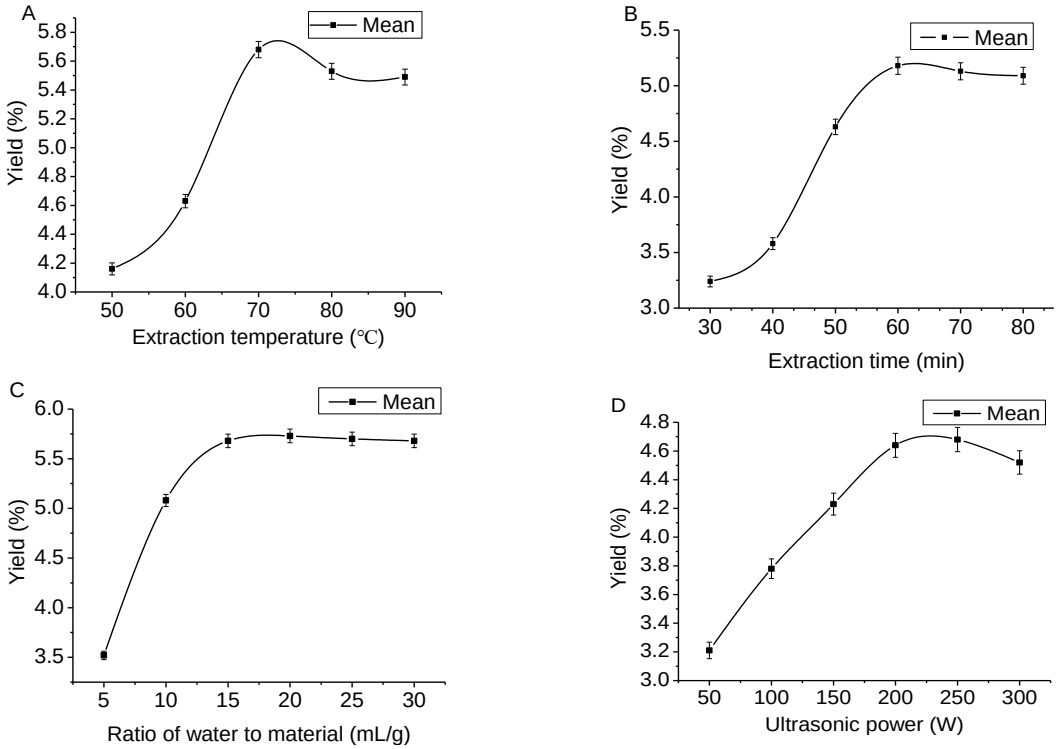

Fig. 1. Effects of different extraction temperature (A), extraction time (B), ratio of water to raw material (C) and ultrasonic power (D) on extraction yield of polysaccharides. All data were mean values of triplicate. The vertical error bars represented the standard deviation of each data point ($P < 0.05$).

Table 2. Box-Behnken design and the response values for the yields of polysaccharides

NO.	X_1 (Extraction temperature, °C)	X_2 (Extraction time, min)	X_3 (Ratio of water to raw material, mL/g)	X_4 (Ultrasonic power, W)	Extraction yield (%)
1	1	1	1	-1	4.11
2	1	1	-1	-1	3.82
3	1	-1	1	1	4.42
4	-1	1	-1	1	4.31
5	1	-1	-1	1	4.26
6	-1	-1	1	-1	4.35
7	-1	1	1	1	4.72
8	-1	-1	-1	-1	4.22
9	-1	0	0	0	4.57
10	1	0	0	0	5.21
11	0	-1	0	0	5.01
12	0	1	0	0	5.17
13	0	0	-1	0	4.73
14	0	0	1	0	5.08
15	0	0	0	-1	4.91
16	0	0	0	1	5.17
17	0	0	0	0	5.17
18	0	0	0	0	5.14
19	0	0	0	0	5.16
20	0	0	0	0	5.15
21	0	0	0	0	5.18

Table 3. Analysis of variance testing the fitness of the regression equation

Source	SS[a]	DF[b]	MS[c]	*F*-value	*p*-value
Model	3.89	14	0.28	133.71	⩽0.0001
X_1	0.20	1	0.20	98.64	⩽0.0001
X_2	0.013	1	0.013	6.16	0.0476
X_3	0.18	1	0.18	86.48	⩽0.0001
X_4	0.034	1	0.034	16.28	0.0068
X_1X_2	0.0007225	1	0.0007225	0.35	0.5768
X_1X_3	0.001013	1	0.001013	0.49	0.5111
X_1X_4	0.022	1	0.022	10.41	0.0180
X_2X_3	0.021	1	0.021	10.12	0.0190
X_2X_4	0.32	1	0.32	151.74	⩽0.0001
X_3X_4	0.002813	1	0.002813	1.35	0.2887
X_1^2	0.26	1	0.26	123.78	⩽0.0001
X_2^2	0.035	1	0.035	16.92	0.0063
X_3^2	0.23	1	0.23	112.36	⩽0.0001
X_4^2	0.071	1	0.071	34.41	0.0011
Residual	0.012	6	0.002076		
Lack of fit	0.011	2	0.005729	22.92	0.0064
Pure error	0.001	4	0.00025		
Cor. total	3.90	20			

[a] Sums of squares [b] Degree freedom [c] Mean square R^2=0.9968 R^2_{adj}=0.9894

Effect of extraction time on extraction yield of polysaccharides The effects of extraction time on the yield of PTGP were shown in Figure 1B. Firstly, the extraction time was set at 30, 40, 50, 60, 70 and 80 min, respectively, while the other extraction parameters were given as the followings: water volume 10 mL, ultrasonic power 150 W and extraction temperature 70 °C. It could be found that the extraction yield increased as extraction time lasted from 30 to 60 min, maximized at 60 min, and then no longer increased.

Effect of ratio of water to raw material on yield of polysaccharides Different ratio of water to raw material could significantly affect the extraction yield. If ratio of water to raw material is too low, polysaccharides in raw material cannot be completely extracted up. On the other hand, high ratio of water to raw material will cause high process

cost [14, 15]. In this study, the effects of ratio of water to raw material on extraction yield of PTGP was investigated. The ratios of water to raw material were set at 5:1, 10:1, 15:1, 20:1 and 25:1, respectively, while other extraction parameters were given as the followings: ultrasonic power 150 W, extraction time 60 min and extraction temperature 70 °C. It could be founded that the extraction yield of PTGP continued to increase obviously with the increasing ratio of water to raw material. But the extraction yield of PTGP started to increase slowly after the ratio of water to raw material exceeded 15:1 (Figure 1C).

Effect of ultrasonic power on yield of polysaccharides Analogously the effects of different ultrasonic power on the yield of PTGP were investigated when the other extraction conditions such as water volume, extraction time, extraction temperature were fixed at 15 mL, 60 min, 70℃respectively. As shown in Figure 1D, the extraction yield of PTGP increased gradually with the increasing of ultrasonic power and reached the maximal value (5.58%) at 200 W, whereas the yield decreased when the ultrasonic power exceeded 200 W.

Optimization of extraction conditions of polysaccharides

Predicted model and statistical analysis Four independent variables including extraction temperature (X_1), extraction time (X_2), ratio of water to raw material (X_3), and ultrasonic power (X_4) were considered and optimized individually using BBD design. Table 2 shows the small design matrix together with the response values obtained. The yield of PTGP ranged from 3.82 to 5.18%, and reached maximum with the ratio of water to raw material of 15 mL/g, at 200 W, 70 °C, and a 60 min treatment duration. Trials No.17-21 in Table 2 were used to determine the experimental error. According to multiple regression analysis on the experimental data, the model for the predicted yield of polysaccharides (Y) could be expressed by the quadratic polynomial equation (in the form of coded values). Statistical testing of the model was performed in the form of analysis of variance (ANOVA). The ANOVA for the fitted quadratic polynomial model of extraction of PTGP was shown in Table 3. The corresponding variables would be more significant if the *F*-value becomes greater and *p*-value becomes smaller [16,17]. Values of *p*-value less than 0.05 showed model terms were significant. From this analysis, the F-value of 133.71 and *p*-value< 0.0001 indicated the response surface quadratic model was significant. The quadratic regression model showed the value of the determination coefficient (R^2) was 0.9968, which implied that 99.68% of the variations could be explained by the fitted model. For a suitable statistical model, R^2_{adj} should be close to R^2. As shown in Table 3, R^2_{adj} was 0.9894, which indicated that only less 1.1% of the total variations could not be explained by the model. It also implied a high degree of correlation between the observed and predicted values. A relatively low value of *C.V.* (coefficient of variation) (0.96%) indicated a better reliability of the experiments values. Significance of the model was also judged by lack-of-fit test. As shown in Table 3, *F*-value and *p*-value of the lack of fit were 22.92 and 0.0064, respectively, which implied that it was significant. The significance of each coefficient was determined using *F*-value and *p*-value. The results were given in Table 3. It could be seen that the independent variables (X_1, X_3, X_4), the interaction terms (X_2X_4) and all two quadratic terms (X_1^2, X_2^2, X_3^2, X_4^2) affected the yield of PTGP very significantly ($p<0.01$), the effects of other independent variables (X_2) and interaction terms (X_1X_4, X_2X_3) was significant too ($p<0.05$). The results also showed that the independent variable X_1 and X_3 were the most significant factor on the experimental yield of PTGP.

Analysis of response surface The relationship between independent and dependent variables was illustrated by the tri-dimensional representation of the response surfaces and the two-dimensional contours generated by the model (shown in Figure 2A-F and 3A-F). Among these four variables (extraction temperature, extraction time, ratio of water to raw material and ultrasonic power), when two variables within the experimental range were depicted in tri-dimensional surface plots, the third variable was kept constant at zero level. The shapes of the contour plots, elliptical or circular, indicated whether the interactions between the corresponding variables were significant or not [16]. An elliptical contour plot means the interactions between the variables are significant while a circular contour plot means otherwise.

Figure 2A and 3A represented the effects of extraction temperature (X_1) and extraction time (X_2) on the yield of PTGP. With the increase of extraction temperature, the yield increased until gradually stable. As far as the effect of extraction time was concerned, the yield initially enhanced as time went by, then decreased slowly. According to the analysis, the optimum PTGP yield should be obtained with an extraction temperature range of 69.5-72.5 °C and an extraction time range of 55.5-60.0 min, respectively, and the effect of extraction temperature was more

significant than that of extraction time. However, the effect of their interaction was not significant.

The effects of extraction temperature (X_1) and ratio of water to raw material (X_3) on the yield of PTGP were shown in Figure 2B and 3B, respectively. It showed that both of the two factors could affect the yield obviously. the production enhanced with the increase of extraction temperature and then remained stable above 70 °C. On the other hand, as the ratio of water to raw material increased from 11.5:1 to 15:1, the yield raised with an increase of extraction temperature from 66.0 to 70 °C.

The conclusions from Figure 2C and 3C demonstrated that extraction temperature (X_1) and ultrasonic power (X_4) represented a significant effect on the yield of PTGP.

When the ratio of water to raw material and extraction time were set at 15:1 and 60 min, respectively, the optimal productivity of PTGP could be gained with an extraction temperature range of 66-70 °C and ultrasonic power range of 185-220 W, and the effect of their interaction on the yield was significant as well as Table. 4 described.

According to Figure 2D and 3D, as the ratio of water to raw material (X_3) increased in the range from 10:1 to 15:1, PTGP yield increased as well. The curve tendency did not level off at low ratio, which indicated that this ratio was well below optimum for PTPG yield. There was a linear increase of the yield with extraction time (X_2) extended from 50 to 60 min, but yet decreased when more than one hour. The effect of extraction time was less significant than the ratio of water to raw material.

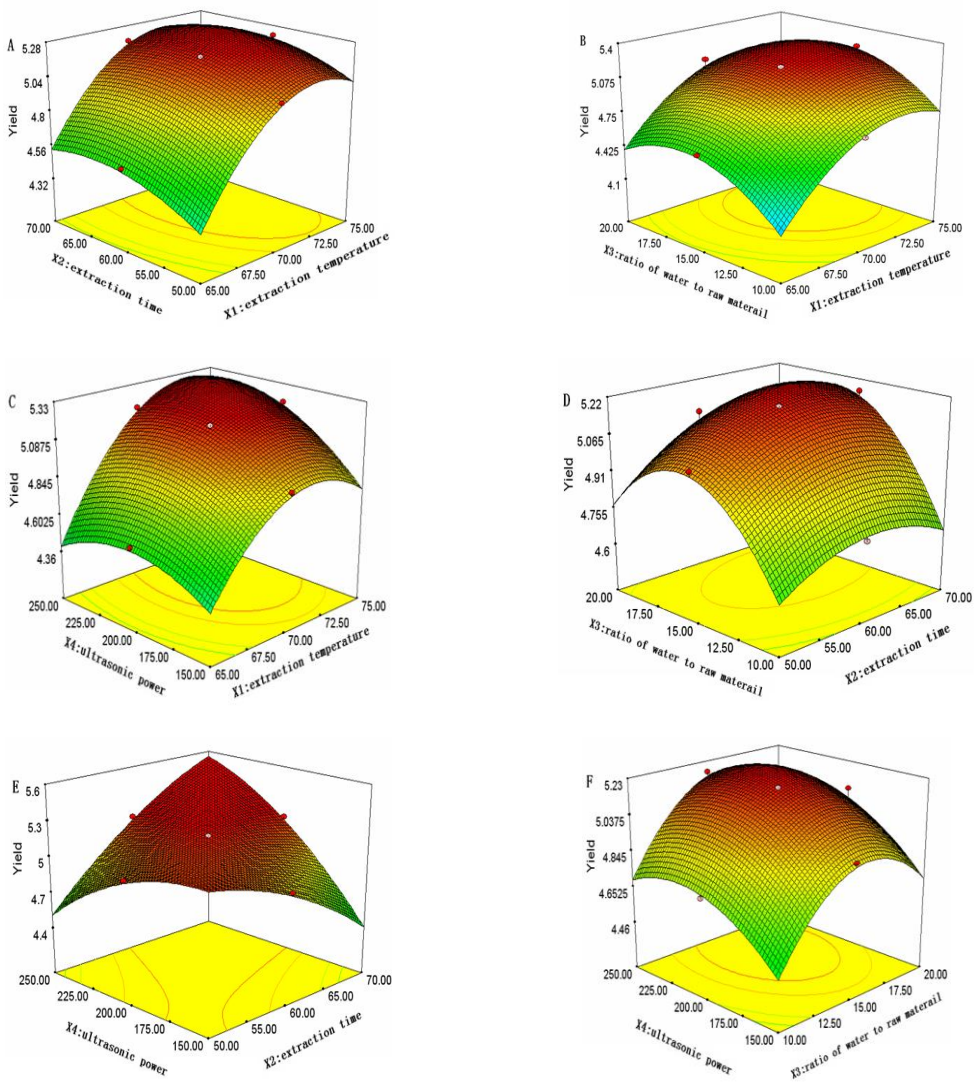

Fig 2. Response surface (3D) showing the effect of different extraction parameters (X_1: extraction temperature, °C; X_2: extraction time, min; X_3: the ratio of water to raw material, mL/g and X_4: ultrasonic power, W) and their interactions on the yield of PTGP.

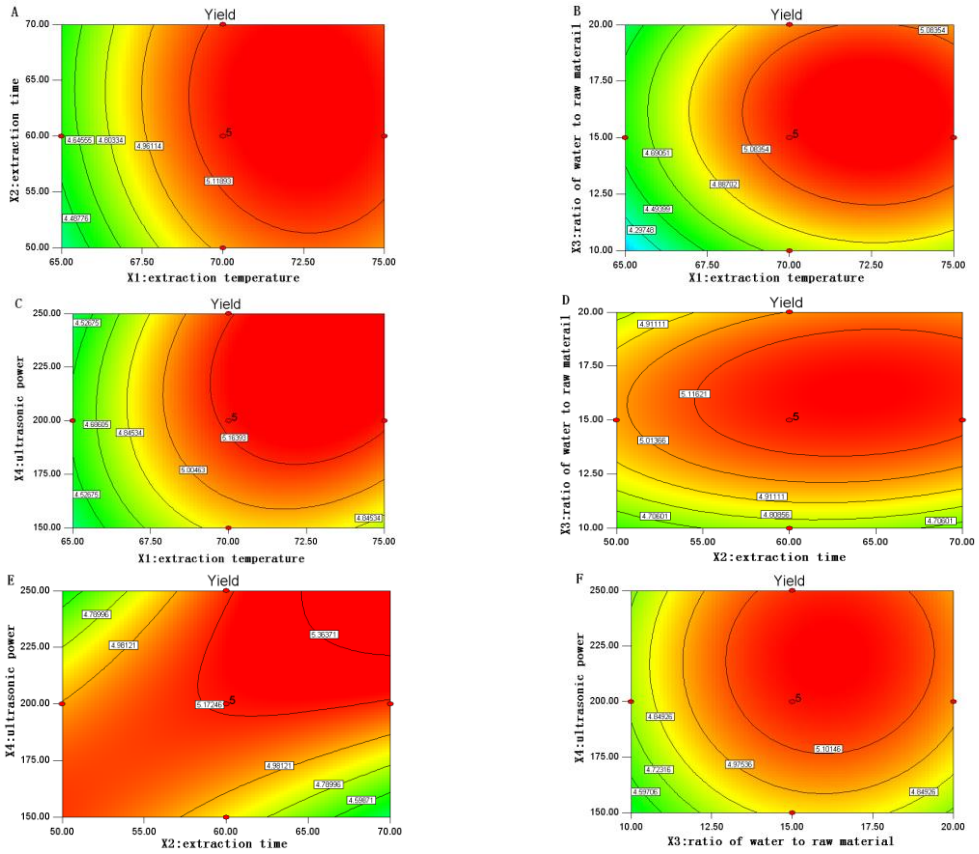

Fig. 3. Contour plots (2D) showing the effect of different extraction parameters (X_1: extraction temperature, °C; X_2: extraction time, min; X_3: the ratio of water to raw material, mL/g and X_4: ultrasonic power, W) and their mutual interactions on the yield of PTGP.

Figure 2E and 3E demonstrated the effects of extraction time (X_2) and ultrasonic power (X_4) on PTGP production. Under the condition of extraction temperature at 70℃and the ratio of water to raw material at 15:1, the yield increased slowly with the extraction time and ultrasonic power increased until up to a threshold level. However, when extraction time and ultrasonic power exceeded the threshold level, the PTGP production decreased due to partial degradation of polysaccharide, which meant that a suitable ultrasonic power was important for extraction of PTGP.

The effects of the ratio of water to raw material (X_3) and ultrasonic power (X_4) on the yield of PTGP could be seen in Figure 2F and 3F. The lower the ratio of water to material, the lower the PTGP yield. The PTGP yield increased obviously with the ratio of water to raw material in the range of 10:1 to 15:1, then changed little. Similarly, the yield enhanced before ultrasonic power increased up to a threshold level of 200 W, but decreased slightly when ultrasonic power was higher than 200 W. These results were consistent with the preliminary experimental results and could determine the accurate value of the parameter.

Optimization of extraction parameters and validation of the model In this study, the model equation for predicting the optimum response values was tested by using the selected optimal conditions. The predicted optimum conditions for polysaccharides extraction and predicted yield were given as follows: extraction temperature of 70.7 °C, extraction time of 66.87 min, the ratio of water to raw material of 12.23 mL/g and ultrasonic power of 232.52 W, respectively. The maximum predicted theoretical yield was 5.25189%. Considering the operability in actual production, the optimal conditions could be modified as follows: extraction temperature of 70.0 °C, extraction time of 66 min, ratio of water to raw material 13 mL /g, ultrasonic power of 230 W. Under this conditions, the mean value of PTGP yield 5.16% ± 1.81% (n=3) was obtained. These results of analysis confirmed that the response model was adequate for the optimization of extraction process, and the model of equation as follows was accurate.

$$Y = 5.19 + 0.32X_1 + 0.08X_2 + 0.13X_3 + 0.13X_4 - 0.021X_{12} - 0.011X_{13} + 0.12X_{14} + 0.051X_{23} + 0.44X_{24} + 0.019X_{34} - 0.32X_1^2 - 0.12X_2^2 - 0.3X_3^2 - 0.17X_4^2$$

FT-IR analysis The FT-IR spectrum of PTGP was shown in Figure 4.

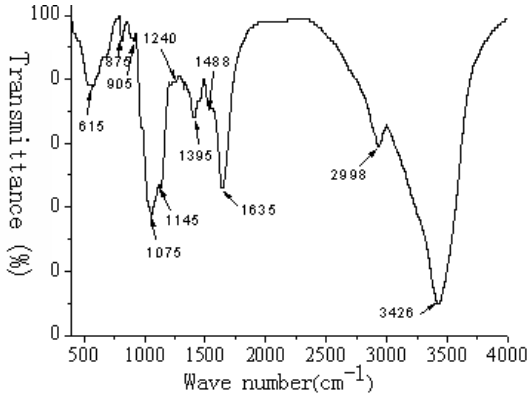

Fig. 4. FT-IR spectrum of PTGP.

A broad band around 3426 cm-1 exhibited O-H stretch vibration [18,19], a weak peak at 2998 cm-1 was assigned to C-H asymmetric stretch vibration [20], a peak at 1635 cm-1 was assigned to the stretching vibrations of the CHO and C=O bonds [18], which was a characteristic absorption band of the bonded water [16,21]. A peak at 1407 cm-1 was regarded as N-H variable angle vibration, which was caused by the -NH3. The peak at 1390 cm-1 was for O-H deformation vibration of CH2O-H. Three stretching peaks at 1240, 1130, and 1060 cm-1 indicated the presence of C-O bonds and pyranose ring in the polysaccharide [22], and the peak broadened due to the C-O stretching vibration. Other stretching peaks 820~950 cm-1 are characteristics absorptions of D-glucose, D-galactose, D-mannose. An obvious absorption peak around 890cm-1 showed that the polysaccharide structure contains β-glucan. These signals all indicated that PTGP had the typical saccharide moiety absorption peaks.

Antioxidant activity of PTGP

Scavenging activity of hydroxyl radical Hydroxyl radicals are highly reactive and undergo chemistry that makes them short-lived. On generation in or exposure of biological systems to these radicals, they can cause damage to cells, including those in humans, where they react with DNA, lipids, and proteins [23]. The result of hydroxyl radical scavenging activities of the polysaccharide was given in Figure 5A, which showed that the difference of scavenging activity between the PTGP with ascorbic acid as a positive control. The scavenging activity increased significantly in a concentration-dependent way at the PTGP concentration range of 0.25-1.5 mg/mL, and achieved maximum scavenging activity of 60.41% at 1.5 mg/mL, which was 30.01% less compared with that of 1.5 mg/mL ascorbic acid (90.42%). Once the value of concentration exceeded 1.5 mg/mL, neither ascorbic acid nor PTGP showed any higher scavenging effects. The results indicated that hydroxyl radicals scavenging activities of PTGP were not obvious at the identical concentration.

Scavenging activity of superoxide radicals The superoxide is a weak oxidant in most organisms, and biologically quite toxic, which could be generated by numerous biological and photo chemical reactions. It could degrade continuously and form other reactive oxygen species (ROS), which contribute to the pathogenesis of many diseases [18]. In this study, scavenging activity of PTGP of superoxide was compared with ascorbic acid, as shown in Figure 5B. The scavenging activity increased gradually along with the concentration. The maximum scavenging ability (89.67%) was obtained at a concentration of 3.5 mg/mL, while ascorbic acid could achieve the maximum scavenging activity of 93.56 % at 1.0 mg/mL. The antioxidant activity of the polysaccharides may be related to monosaccharide component, molecular size, structure and conformation [19, 24].

Scavenging activity of DPPH radicals The methods of scavenging DPPH radical are well acknowledged and widely applied to determinate the free radical scavenging ability of many antioxidants [25, 26, 27]. In this work, DPPH free-radical scavenging effect of PTGP and ascorbic acid were measured respectively as shown in Figure 5C. At the concentration from 0.25 mg/mL to 4.0 mg/mL, the DPPH radical scavenging activity increased with the PTGP concentration until the maximum value of 61.23 % at 3.0 mg/mL. Whereas the maximum scavenging rate of ascorbic acid could reach 84.93 % at 1.0 mg/mL. PTGP scavenging activity of DPPH radicals was not as strong as ascorbic acid.

Reducing power The reducing power, which is regarded as the other one of significant activity indexes, could be assessed by a Fe^{3+}-Fe^{2+} reduction reaction, and is stronger with higher absorbance value in this reaction [18]. The reducing power of PTGP was shown in Figure 5D. The maximum reducing power of ascorbic acid and PTGP were obtained at 0.50 mg/mL (absorbance=1.3226) and 3.0 mg/mL (absorbance=0.5867), respectively.

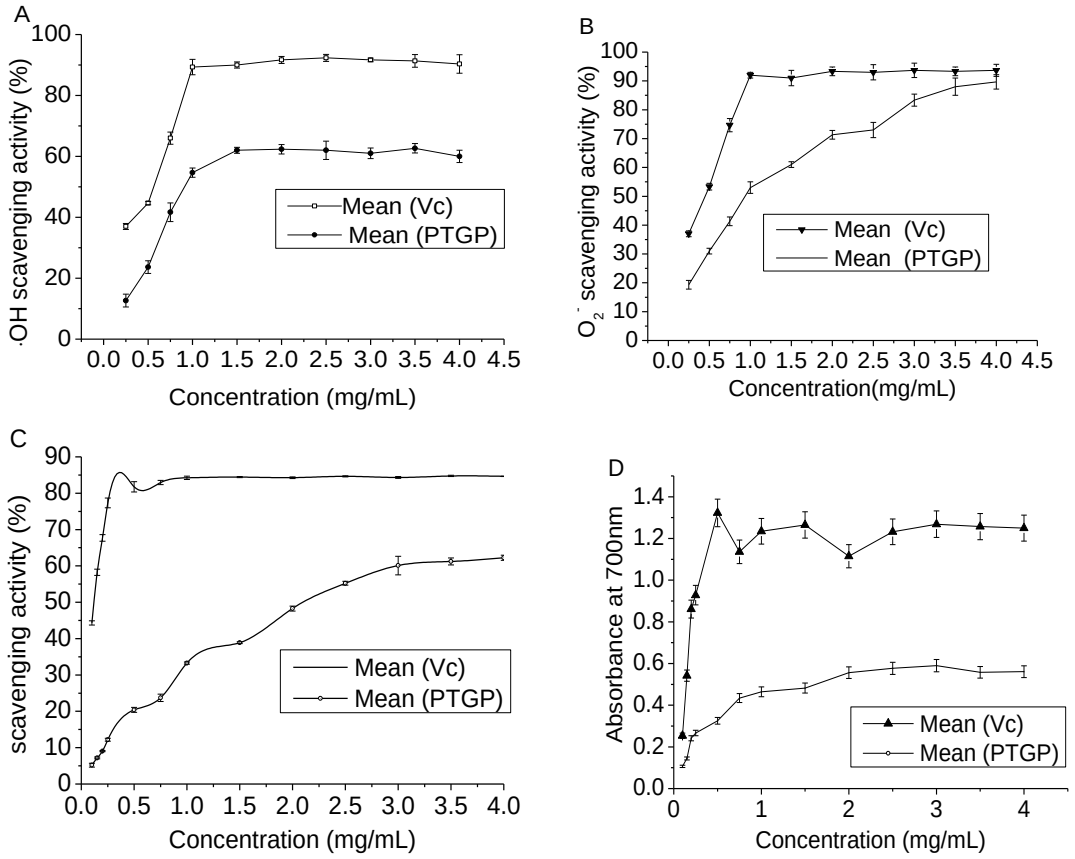

Fig. 5 Antioxidant activities of PTGP. (A) Scavenging ability of hydroxyl radicals. (B) Scavenging ability of superoxide anion. (C) Scavenging activity of DPPH radical. (D) Reducing power. Data were shown as mean (n=3).The vertical bars represented the standard deviation of each data point ($P < 0.05$).

CONCLUSION

Ultrasonic-assisted extraction procedure was used to extract the crude polysaccharides from Piteguo fruit. And Box-Behnken design (BBD) [7] was introduced to optimize the ultrasonic-assisted extraction technology condition. The structures and activities of polysaccharide were analyzed. The results shown the optimized extraction conditions as follows: ratio of water to raw material 13:1 mL/g, extraction time 66 min, extraction temperature 70 °C, and ultrasonic power 230 W, and the extraction ratio of crude PTGP was up to 5.16% ± 1.81%, which is was well matched with the value (5.25%) predicted by the BBD model. PTGP was composed of Glc with β-type pyranoid type sugar ring, displayed certain antioxidant ability and could be used as a potential natural antioxidant.

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ИЗСЛЕДВАНЕ НА ЕКСТРАКЦИЯТА И АНТИОКСИДАНТНАТА АКТИВНОСТ ПОЛИЗАХАРИДИ ОТ ПЛОДОВЕ НА *Piteguo*

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(Резюме)

Изследвана е ултразвуковата екстракция (UAE) на полизахариди от плодовете на *Piteguo* (*Pyrus sinkiangensis*). Четири параметъра (отношението вода/твърда фаза, температура, време на екстракция и мощност на ултразвука) са оптимизирани по плана на Box-Behnken (BBD). Структурата на полизахаридите са предварително анализирани по инфрачервените им спектри. Открита е *in vitro* антиоксидантна активност. Резултатите показват, че екстракционното отношение на суровия плод е до 5.16% ± 1.81% под оптималните условия за екстракция: отношение вода/твърда фаза 13:1 mL/g, време за екстракция 66 мин, температура 70°C и мощност на ултразвука 230 W, което добре се съгласува със стойността (5.25%) предвидена от модела BBD. Спектроскопското изследване показва, че плодовете съдържат съединения Glc с β- пираноиден пръстен. Освен това тестовете показват известна антиоксидантна активност и че плодовете може да се използват като потенциални антиоксиданти.