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ORIGINAL RESEARCH PAPER

ALKALI EXTRACTION OF POLYSACCHARIDES FROM PROSO MILLET AND ANTIOXIDANT ACTIVITY

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Abstract: The alkali extraction of polysaccharides from proso millet was investigated by Response Surface Methodology (RSM) based on a three level, four variable Box–Behnken designs in order to obtain an optimal extraction condition combination of alkali concentration, liquid-solid ratio, extraction time, and extraction temperature. The experimental results were fitted to a quadratic polynomial and analyzed. Meanwhile was the antioxidant activity of polysaccharides extracted from proso millet measured in vitro. The results showed that the optimum conditions were alkali concentration 0.87 mol·L^{-1}, liquid-solid ratio 23.1:1 mL·g^{-1}, extraction time 1.61 h and extraction temperature 77.7 °C, at this condition combination, the experimental yield of polysaccharides was 38.27 ± 0.35 mg·g^{-1}, which was very close to the model predicted value of 36.12 mg·g^{-1}. The quadratic polynomial mathematical model had a good degree of fitting with experimental data ($p < 0.0001$) and could there out indicate the optimal extraction process conditions. The proso millet polysaccharides were proved by the antioxidant activity experiment to have better radical scavenging activity on superoxide, hydroxyl and DPPH (1,1-Diphenyl-2-picrylhydrazyl).

Keywords: *alkali extraction, antioxidant activity, polysaccharides, proso millet, response surface methodology*

INTRODUCTION

The proso millet (*Panicum miliaceum* L.) is an annual grass species used as a warm-season crop, has well ability to adapt to the soil and climatic conditions; its growth period is short and the demand for water is little. Its seeds can be used as food, therefore it is far and wide planted in the arid region of China, India, Nepal, the Middle East, Russia, Belarus, Turkey, Ukraine and Romania. The seeds have rich in starch, dietary fiber, protein, fat, vitamin and trace elements. It has a number of functionalities salutary to human health, for instance the precaution and supervision of diabetes mellitus [1], callus and shoots regeneration from protoplasts of proso millet [2]. The researches demonstrated that the polysaccharides have distinct biological properties such as anti-viral, anti-oxidative and anti-complementary activities [3 – 6]. They play an important role in the process of living organism's growth and development [7]. Because of its effective to optimize process variables, Response Surface Methodology (RSM) has extensive use in optimization process variables of the effective component extraction from various biomasses [8 – 12].

It was found that there was little reports in regard to the optimization of alkali extraction of proso millet polysaccharides (PMP) with RSM and research of antioxidant activity in vitro of PMP through consulting literature, although there were many reports of proso millet study results [1, 2, 13]. In this study, the alkali extraction experiments and the test of antioxidant activity in vitro of PMP were implemented. Once extracted and 150 rpm stirring rate in all extraction experiments, the influence of alkali concentration (sodium hydroxide aqueous solution, $mol \cdot L^{-1}$), liquid-solid ratio (alkali aqueous solution's volume with foxtail millet particle's mass, $mL \cdot g^{-1}$), extraction time (h), and extraction temperature (°C) on the PMP yield were respectively investigated, then RSM was used to optimize extraction conditions and to roundly evaluate the influence degrees of extraction parameters on the yield of PMP and interaction between them [14]. The antioxidant activity of PMP in vitro, i.e. radical scavenging activities on superoxide, hydroxyl and DPPH (1,1-Diphenyl-2-picrylhydrazyl), was further researched to appraise their biological activity.

MATERIALS AND METHODS

Materials and Extraction of Polysaccharides

The unshelled product of proso millet seed was obtained from Donghua rice industry limited company, Jinzhou, Liaoning, China, 2016 of harvest. The grain diameter was about 2 - 3 mm. Sample needed to pretreatment, namely was first put into digital constant temperature drying oven (WX881-TG, Wujiang City Prestige Electric Equipment CO., LTD, Jiangsu, China) to dry at 60.0 °C until constant mass. Five grams of dry sample was mixed different volume of different concentration alkali solution, and extracted in a 500 mL three-neck flask, then it was immersed in hot water of digital electrically - heated thermostatic water bath (HH-S4, Jintan City Jinnan Instrument Manufacturing CO., LTD, Jiangsu, China) controlled the needed extraction temperature, the suspension liquid in flask was immediately stirred at 150 rpm with a constant speed stirrer (S-212, Shanghai Shensheng Bio-Tech CO., LTD, Shanghai, China) and

extracted a set time. After extraction operation, the mash was quickly put in cold bath, cooled down to room temperature and then vacuum-filtered. The obtained filtrate was put into a rotary evaporator (YRE-201D, Gongyi City Yuhua Instrument CO., LTD, Henan, China) concentrated to 20 % of initial volume under the vacuum state. Four volumes of dehydrated ethanol was put into the obtained concentrate, mixed into solution which the ethanol final concentration was 80 % in order to alcohol-precipitate the PMP. The obtained suspension solution was then centrifuged at 3000 rpm for 10 minutes using centrifuge (TGL-16G, Changzhou Meixiang Instrument CO., LTD, Jiangsu, China), the supernatant fluid was removed and the precipitate was retain, then the dehydrated ethanol was used to wash the precipitate three times. The extract was dried at 50 °C in oven until constant mass, and then weighted. Sampled, dissolved and fixed volume for extractive, and then the concentration of PMP content was examined with a spectrophotometer (T-6v, Nanjing Feile Instrument CO., LTD, Jiangsu, China) [14]. The sample was only extracted once and the extraction experiment of the same conditions was repeated twice. All reagents were of analytical grade.

Experimental Design

The single factor experiments were first implemented, the alkali concentration changed from 0.2 to 1.0 mol·L^{-1}, the range of liquid-solid ratio was 10.0:1-30.0:1 mL·g^{-1}, the extraction time altered from 0.5 to 2.5 h and the extraction temperature scope was 50.0 - 90.0 °C, then based on the single factor experimental results, a three-level, four-variable Box-Behnken Design was used in ascertaining the optimum combination of alkali extraction conditions for acquiring highest PMP yield [14]. Four independent variables pondered in extraction process were, alkali concentration (mol·L^{-1}, X_1), liquid-solid ratio (mL·g^{-1}, X_2), extraction time (h, X_3), and extraction temperature (°C, X_4) [14 – 16]. The PMP yield was used as the dependent variable. The full experiment scheme was consists of 29 experimental points (incorporating five repetitive experiments at the center point). A quadratic polynomial mathematical model was used to relate independent variables and dependent variable and the regression coefficients were gotten by statistical analyses. The relationship between independent variables and dependent variable could be expressed in the following equation 1:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i \neq j=1}^{3} \sum_{j=1}^{4} \beta_{ij} X_i X_j \quad (1)$$

where Y is the obtained response value (PMP yield) of each experiment, mg·g^{-1}; β_0, β_i, β_{ii}, β_{ij} are constant regression coefficients of equation 1; X is the independent variables code levels; X_iX_j and X_i^2 express the interaction and quadratic terms of independent variables, respectively [14 – 16].

Analysis of Samples

The phenol-sulphuric acid colorimetric method at 485 nm was applied to measure the PMP content [14, 17]. In this method the glucose was used as standard, the PMP yield could be obtained through calculating with equation 2:

$$Y = \frac{X \times V}{M} \times 1000 \quad (2)$$

where Y, V, M and X is the PMP yield, mg·g^{-1}; the total volume after the crude PMP dissolved and metered volume, L; the raw material consumption of proso millet, g; and the determined PMP concentration in solution at 485 nm, g·L^{-1}; respectively.

Assay for Antioxidant Activity

Superoxide radical scavenging

The scavenged of superoxide radical by antioxidants was appraised by the inhibition of pyrogallol acid as the description in the previous [18]. In a nutshell, 4.5 mL of 50 mM Tris–HCl buffer (*p*H 8.2) was mixed with 4.2 mL of deionized water. After incubation at 25 °C for 20 min, 1 mL of PMP solution (0.2 - 1.0 mg·mL^{-1}) and 0.4 mL of pyrogallic acid were put into the mixed solution. The obtained mixture was promptly wobbled and fostered at 25 °C for 5 minutes. Where after, 8 mM HCl was put into the mixed solution to stop the reaction, and its absorbancy was measured at wavelength 320 nm. Ascorbic acid was used as a positive control. The ability of PMP to scavenge superoxide radicals was determined with equation 3:

$$\text{scavenging activity}(\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100 \quad (3)$$

where A_0, A_1 is the blank absorbance, the absorbance of PMP/Vc, respectively.

Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radical was observed according to the method of Li et al [19]. The reaction mixed solution (2.5 mL) included 0.5 mL of $FeSO_4$ (1.5 mM), 0.15 mL of sodium salicylate (20.0 mM), 0.35 mL of H_2O_2 (6.0 mM), and 1.0 mL of test sample solution set different polysaccharides concentrations (0.2–1.0 mg·mL^{-1}). After incubation for 60 minutes at 37 °C, the absorbance of the formed hydroxylated salicylate complex substance was gauged at wavelength 562 nm with ascorbic acid as the positive control. The percentage of hydroxyl radical scavenging effect was calculated according to Equation 4:

$$\text{hydroxylradical-scavenging activity}(\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \quad (4)$$

where A_0, A_1, A_2 is the absorbance of the solvent control, the sample or ascorbic acid, the reagent blank without sodium salicylate, respectively.

Radical scavenging activity on DPPH

The procedure narrated by Zhang et al. [20] was used to measure the DHHP free radicals scavenging activity of the prepared PMP. Briefly, 2 mL of 0.1 mM DPPH ethanol solution prepared freshly was put into 1 mL of various concentrations (0.2-1.0 mg·mL^{-1}) of the PMP sample solution. After the mixtures was wobbled and incubated at 25 °C for 30 minutes, the absorbance of sample mixtures was measured at wavelength 517 nm with ascorbic acid as a positive control at the same concentration. The radical scavenging activity on DPPH was calculated using Equation 5:

$$\text{DHHP scavenging activity}(\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100 \quad (5)$$

where A_0, A_1 is the absorbance of mixture solution without sample, the test sample mixed with reaction solution, respectively.

Statistical Analyses

The Design Expert Software was applied to multiple nonlinear regressions of the responses gotten from each design experiment. The fitting degree of between the experimental data and the equation was inspected using the coefficient of determination R^2, F-test and p-value were used for checking the significance level of the regression coefficient. p-value below 0.05 was regarded as statistically significant [14].

RESULTS AND DISCUSSION

Investigation of Single Factor

Effects of alkali concentration on the yield of polysaccharides

Alkali can help exclude the physical and chemical impact between cell wall of polymer molecules, make more polysaccharides to dissolve from cells into solution, for this reason the polysaccharides yield is increased, whereas too high alkali concentration would bring about the reducing of the polysaccharides yield as a result of the polysaccharides structure breakage aroused by the alkali catalyzed hydrolysis [15, 16]. The effect of different alkali concentration on the PMP yield was shown in Figure 1a. Extraction experiment was carried out at different alkali concentration conditions while other extraction variables were set as follows: liquid-solid ratio 20.0:1 $mL \cdot g^{-1}$, extraction time 1.0 h, and extraction temperature 70.0 °C. When alkali concentration varied from 0.2 to 0.8 $mol \cdot L^{-1}$ the yield increased relaxedly, it reached a maximum at 0.8 $mol \cdot L^{-1}$, and then decreased rapidly along with increasing alkali concentration. This indicated that alkali concentration of 0.8 $mol \cdot L^{-1}$ was sufficient to obtain the PMP production.

Effects of liquid-solid ratio on the yield of polysaccharides

The effect of different liquid-solid ratio on the PMP yield at alkali concentration 0.8 $mol \cdot L^{-1}$, extraction time 1.0 h and extraction temperature 70.0 °C was represented in Figure.1b. The PMP yield raised obviously with the increase of liquid-solid ratio from 10.0:1 to 20.0:1 $mL \cdot g^{-1}$, the yield achieved a peak value at 20.0:1 $mL \cdot g^{-1}$, because of the raised of liquid-solid ratio leading to the augment of mass transfer driving force [21]. However, the yields decreased slightly with the ratio increasing continuous, due to the increase of amount of alkali in the extract system with the increase of liquid-solid ratio leading to degraded of extracted PMP, the large liquid-solid ratio might meanwhile cause an increase of process operating cost [17]. These results were similar to the earlier observation [16, 22]. Thus, liquid-solid ratio 20.0:1 $mL \cdot g^{-1}$ was favorable for producing the PMP.

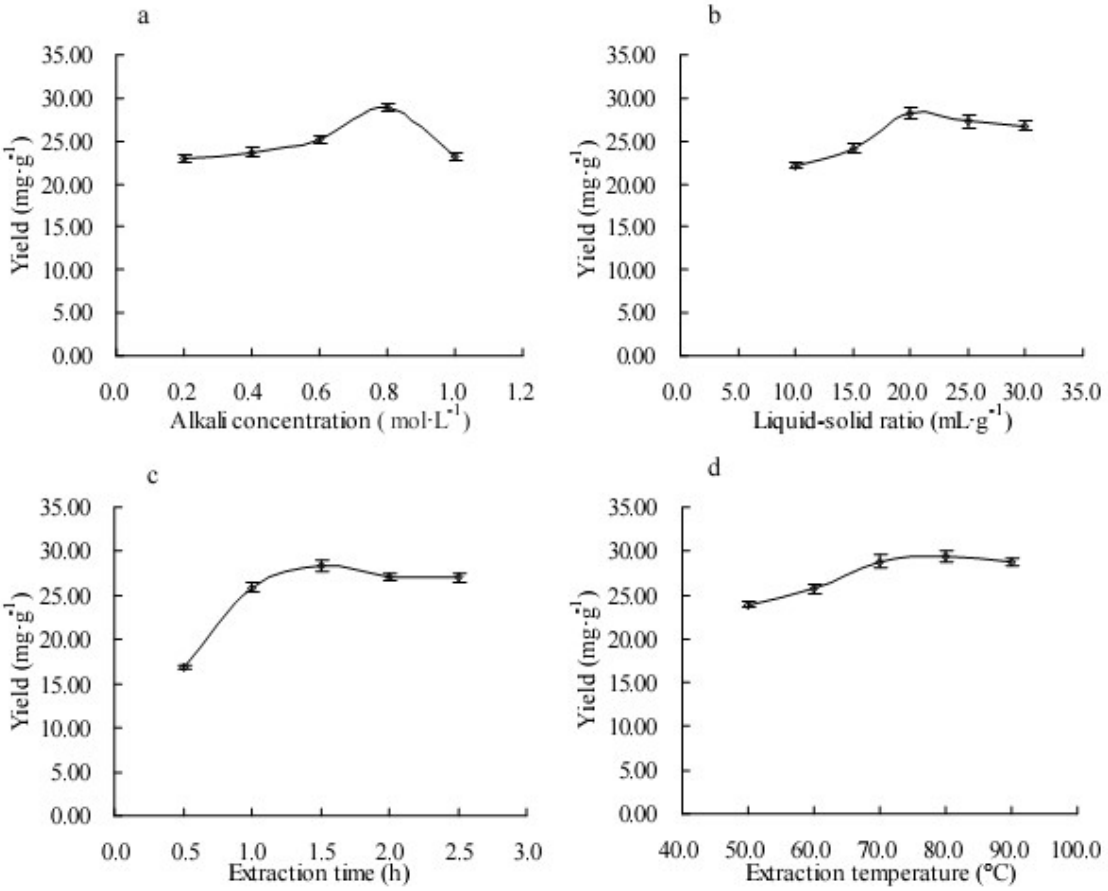

Figure 1. *Effects of four factors on extraction yield of polysaccharides: (a) acid concentration, (b) liquid-solid ratio, (c) extraction time, (d) extraction temperature*

Effects of extraction time on the yield of polysaccharides

The effects of different extraction time on the PMP yield were investigated while alkali concentration, liquid-solid ratio and extraction temperature were fixed at 0.8 mol·L^{-1}, 20.0:1 mL·g^{-1} and 70.0 °C, respectively. Figure 1c showed that the PMP yield obviously increased with extraction time, and achieved the maximum value at 1.5 h, this was possible because the processes of broken the proso millet cell-wall, penetrating of liquid into proso millet particle interior, dissolved of the polysaccharides and where after diffusing out from particle interior to solvent required certain time. Hour and Chen [23] reported that the polysaccharides production process needed a long extraction time. But the PMP yield fell down distinctly after 1.5 h. It was conjectured that polysaccharides dissolved in alkali solution was hydrolyzed because of a long time in solution [24]. 1.5 h was hence selected to be optimal extraction time in the present experiment.

Effects of extraction temperature on the yield of polysaccharides

The implementation of extraction experiment was under 50.0, 60.0, 70.0, 80.0 and 90.0 °C, respectively, and other extraction conditions were as following: alkali concentration 0.8 mol·L^{-1}, liquid-solid ratio 20.0:1 mL·g^{-1} and extraction time 1.5 h. The effects of different temperature on the PMP yield were represented in Figure 1d. As shown in Figure 1d, the PMP yield observably improved from 50.0 to 70.0 °C, and then raised rarely from70.0 to 80 °C, the maximum yield was observed at 80.0 °C, but the

yield was slightly decreased after 80.0 °C. It was that with the increase of temperature, the solvent viscosity was decreased to improve the solvent and solute diffusivity in the suspension solution system, which improved the dissolving capacity of polysaccharides. However, too high temperature would lead to degradation of polysaccharides [16, 25]. This tendency was the same as literature report of other author in extracting polysaccharides [26]. Therefore, 80.0 °C was used as optimal extraction temperature.
Based on the single factor experiment results, the range of 0.6 - 1.0 mol·L^{-1} alkali concentration, 15.0 - 25.0 mL·g^{-1} liquid-solid ratio, 1.0 - 2.0 h extraction time and 70.0 - 90.0 °C extraction temperature (Table 1) were adopted in RSM experiments.

Table 1. *Experimental design and results of alkali extraction*

Test number	Alkali concentration X_1 [mol·L^{-1}]	Liquid-solid ratio X_2 [mL·g^{-1}]	Extraction time X_3 [h]	Extraction temperature X_4 [°C]	Y [mg·g^{-1}]
1	1.0	20.0:1	1.0	80.0	29.82±0.38
2	0.8	25.0:1	1.5	70.0	33.20±0.41
3	0.6	25.0:1	1.5	80.0	32.09±0.46
4	0.8	25.0:1	1.0	80.0	30.60±0.39
5	1.0	20.0:1	2.0	80.0	31.27±0.48
6	0.8	20.0:1	1.0	90.0	27.06±0.35
7	0.8	15.0:1	2.0	80.0	25.40±0.37
8	0.8	15.0:1	1.5	70.0	27.24±0.51
9	0.6	20.0:1	1.5	70.0	30.41±0.45
10	0.6	20.0:1	1.0	80.0	31.56±0.52
11	1.0	20.0:1	1.5	70.0	33.93±0.56
12	1.0	20.0:1	1.5	90.0	28.70±0.43
13	0.6	20.0:1	1.5	90.0	30.52±0.38
14	0.8	25.0:1	2.0	80.0	32.78±0.61
15	0.8	25.0:1	1.5	90.0	33.24±0.50
16	0.8	20.0:1	2.0	90.0	29.46±0.33
17	0.8	15.0:1	1.0	80.0	27.66±0.36
18	0.6	20.0:1	2.0	80.0	29.56±0.44
19	0.8	20.0:1	2.0	70.0	31.01±0.52
20	1.0	25.0:1	1.5	80.0	34.35±0.49
21	1.0	15.0:1	1.5	80.0	25.25±0.29
22	0.8	15.0:1	1.5	90.0	26.88±0.32
23	0.6	15.0:1	1.5	80.0	26.59±0.22
24	0.8	20.0:1	1.0	70.0	28.42±0.43
25	0.8	20.0:1	1.5	80.0	34.88±0.46
26	0.8	20.0:1	1.5	80.0	34.50±0.46
27	0.8	20.0:1	1.5	80.0	35.81±0.46
28	0.8	20.0:1	1.5	80.0	35.16±0.46
29	0.8	20.0:1	1.5	80.0	34.69±0.46

The Results of Optimization by RSM

Model fitting

The factors and their levels of alkali extraction process were chosen on the basis of single-factor experiments (Table 1). The experiment design proposed project and the responsive value of PMP yield were collected in Table 1. The regression analysis results were listed in Table 2. The PMP yield response value Y was expressed by the nether (Equation 6) in terms of actual values:

$$\begin{aligned} Y = & -232.014 + 98.747X_1 + 3.775X_2 + 22.427X_3 + 4.212X_4 + 0.899X_1X_2 \\ & + 8.610X_1X_3 - 0.669X_1X_4 + 0.444X_2X_3 + 2.475X_2X_4 - 9.600X_3X_4 \\ & - 46.915X_1^2 - 0.118X_2^2 - 12.232X_3^2 - 0.024X_4^2 \end{aligned} \quad (6)$$

Table 2. *Analysis of variance for regression equation to alkali extraction*

Source	Sum of Squares	df	Mean	*F* Value	Prob＞*F*	significance
Model	258.54	14	18.47	15.91	<0.0001	‡
X_1	20.52	1	20.52	17.68	0.0009	‡
X_2	6.14	1	6.14	5.29	0.0373	†
X_3	3.25	1	3.25	2.80	0.1165	*
X_4	2.86	1	2.86	2.47	0.1386	*
X_1X_2	3.23	1	3.23	2.79	0.1173	*
X_1X_3	2.97	1	2.97	2.55	0.1323	*
X_1X_4	7.16	1	7.16	6.16	0.0263	†
X_2X_3	4.93	1	4.93	4.24	0.0585	*
X_2X_4	0.061	1	0.061	0.053	0.8216	*
X_3X_4	9.216E-0.03	1	9.216E-0.03	7.939E-0.03	0.9303	*
X_1^2	22.84	1	22.84	19.68	0.0006	‡
X_2^2	56.84	1	56.84	48.96	<0.0001	‡
X_3^2	60.66	1	60.66	52.26	<0.0001	‡
X_4^2	36.23	1	36.23	31.21	<0.0001	‡
Residual	16.25	14	1.16			
Lack of Fit	15.21	10	1.52	5.82	0.0521	
Pure Error	1.044	4	1.16			
Cor Total	274.79	28				
R^2 0.9409 Adj. R^2 0.8817						

* Not significant; † Significant; ‡ Extremely significant; df, degrees of freedom.

The analysis results of variance (ANOVA), goodness-of-fit and the adequacy of the model were collected in Table 2. The p-value was less than 0.05, which manifested that the selected variables and their scope investigated had notable effect on the PMP yield. Then the residual analysis was implemented to verify the adequacy of the obtained model and ensure whether the approximating model would cause poor or misleading results [27]. Linear term of X_1 (alkali concentration, p = 0.0009) was extremely significant effect on PMP yield. Each quadratic term (p < 0.05) was also extremely significant. Linear term of X_2 (liquid-solid ratio, p = 0.0373) and the interaction term of X_1 and X_4 (acid concentration and extraction temperature, p = 0.0263) were significant. The other terms were however not significant. The p -value of model was less than

0.0001 and Adj. R^2 was 0.8817 which would give a better fit to the mathematical model (Equation 6). Figure 2 showed the residual and the influence plots for the experimental data [14, 27].

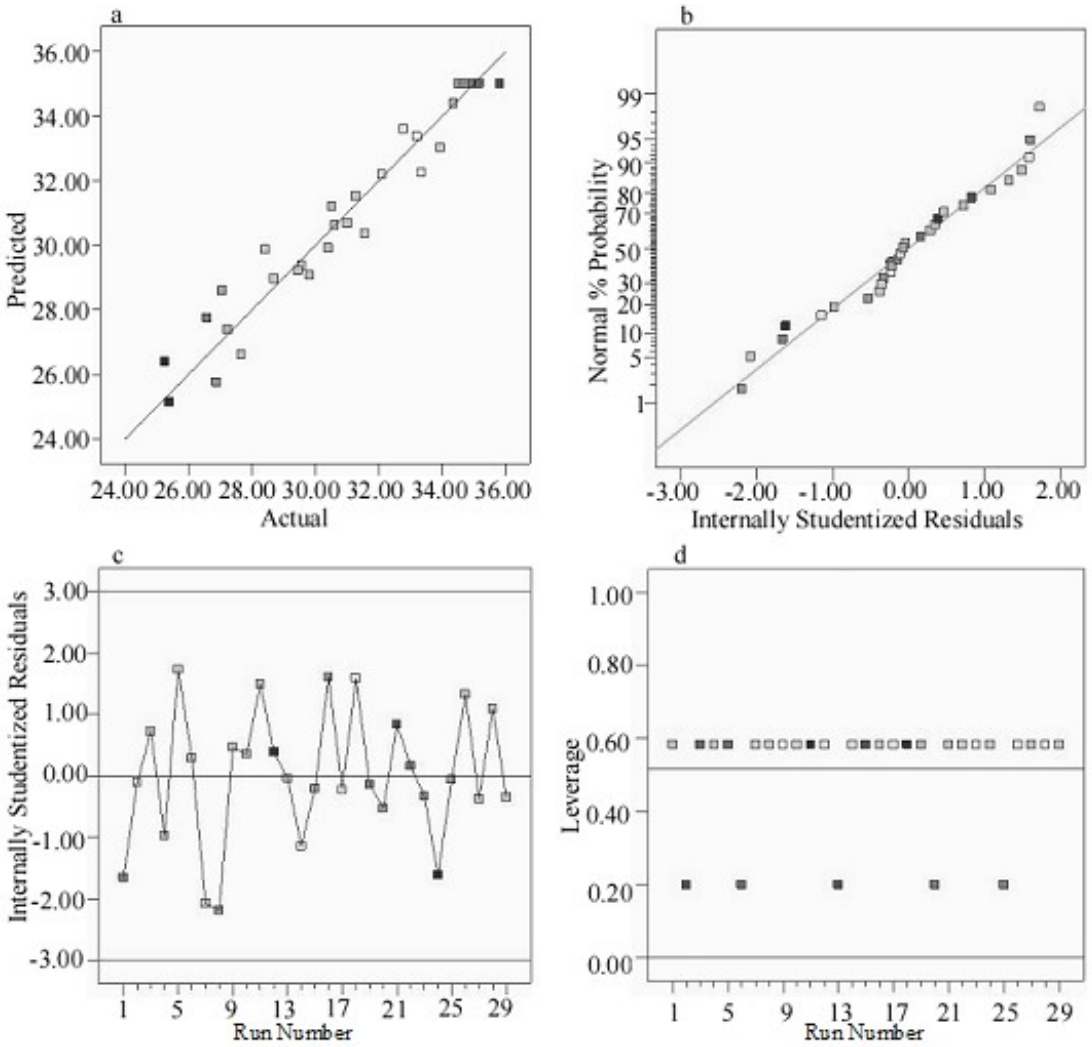

Figure 2. *Diagnostic plots for the model adequacy of alkali extraction (a) Predicted vs. Actual, (b) Normal plot of Residuals, (c) Residuals vs. Run, and (d) Leverage vs. Run*

The predicted values were fully close to the practical values, all the predicted points and experimental points were better located to the 45° line (Figure 2a), pointing that the model obtained could successfully uncover the relevance between the process variables on the response. The normal % probability plot of residuals for response shown in Figure 2b was normally distributed, as they located tightly to a straight line and showed no deviation of the variance existing. The good fit of the model was explained by establishing the relationship of internally studentized residuals versus predicted and showed that all the data points located within the limit values (Figure 2c). Because all leverage values were less than 1 (Figure 2d), there were no outliers or unexpected errors

in the model. The above analytic results demonstrated a good relationship between BBD experimental data and the model could be better used to predict yield of PMP.

Verification of the model

The optimized conditions of alkali extraction of PMP predicted by equation 6 were alkali concentration 0.87 mol·L^{-1}, liquid-solid ratio 23.06:1 mL·g^{-1}, extraction time 1.61 h and extraction temperature 77.73 °C. At this conditions, the PAM yield was predicted by equation 6 for 36.12 mg·g^{-1}.

The experiment were repeated three times at 0.87 mol·L^{-1} of alkali concentration, 23.1:1 mL·g^{-1} of liquid-solid ratio, 1.61 h of extraction time and 77.7 °C of extraction temperature. The mean value of actual PMP yield was 38.27 ± 0.35 mg·g^{-1} (n = 3), the relative error was 5.95 % compared with indicated value of 36.12 mg·g^{-1}. The experimental results explained further that equation 6 was suitable for reflecting the anticipative optimization, and was relatively accurate and satisfactory.

Assay for Antioxidant Activity

Superoxide radical scavenging

The superoxide radical, one of the precursors of singlet oxygen and hydroxyl radicals, indirectly starts lipid peroxidation. The superoxide anions can as well deteriorate cellular damage because of their capacity to generate other types of free radicals and oxidants [18]. Scavenging activity of PMP on superoxide radicals was presented in Figure 3a. It was obviously that the scavenging ability of PMP on superoxide radicals correlated positively well with increasing concentrations of PMP in range of investigated concentration, but this increase became less obvious when the concentration approached 1.0 mg·mL^{-1}. Results demonstrated that PMP had a noticeable effect on inhibiting the formation of superoxide radical especially at high concentrations, although the superoxide radical scavenging rate of PMP was lower than that of ascorbic acid. Similar results have been reported in other plant polysaccharides [18, 28].

Hydroxyl radical scavenging activity

It is significant to wipe out hydroxyl radicals for antioxidant protection because hydroxyl radicals are one of the active oxygen variety produced in the body, which can easily cross cell membranes, promptly react with most biomolecules, and inflict tissue damage or cell death [19, 28]. As shown in Figure 3b, the PMP exhibited a concentration-dependent hydroxyl scavenging activity in range of investigated concentration and high hydroxyl scavenging activity. Results indicated that PMP had a high level of hydroxyl radical scavenging effect, but the scavenging ability of PMP on hydroxyl radicals lower than that of the ascorbic acid. The results were universally similar to the literature on the studies of various polysaccharides antioxidant activity [20, 28].

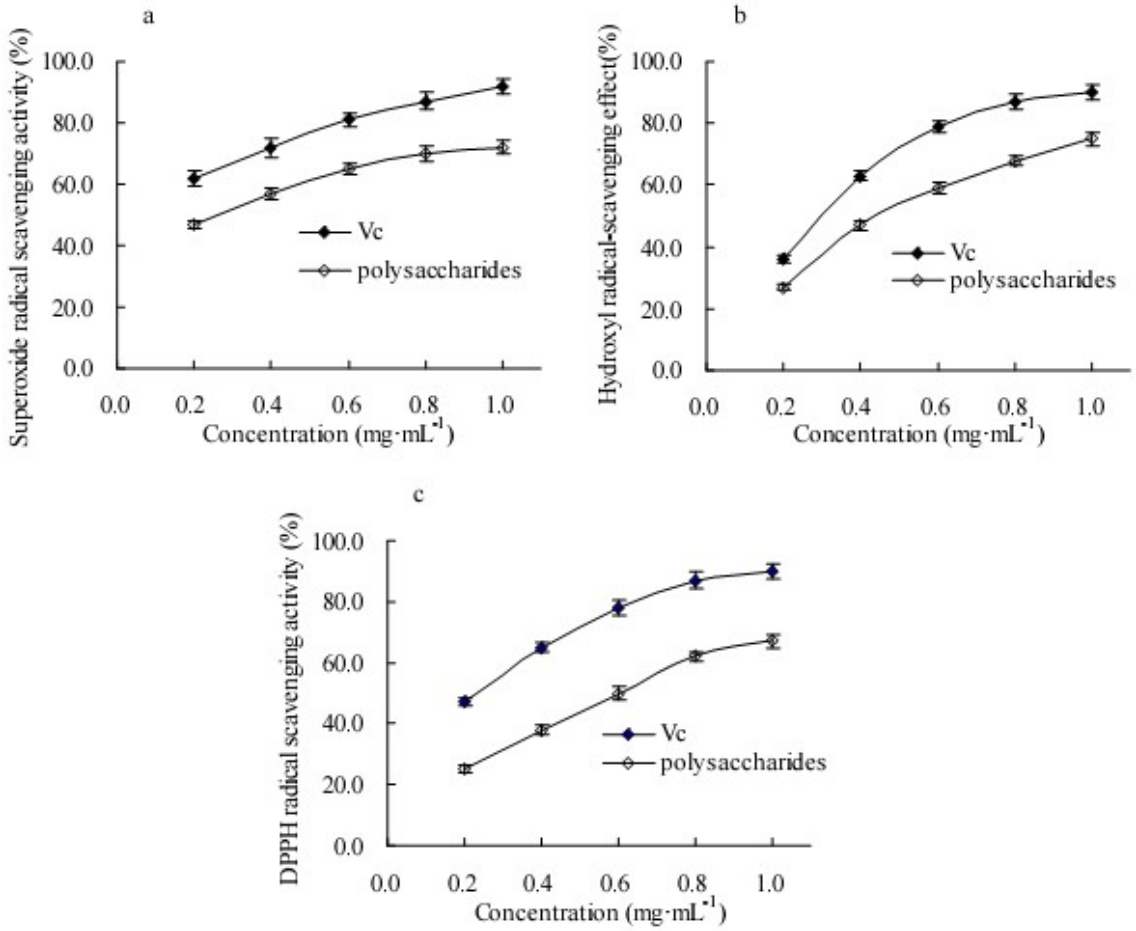

Figure 3. *Radical-scavenging activities (%) of the polysaccharides and ascorbic acid on (a) Superoxide anion, (b) Hydroxyl, and (c) DPPH*

Radical scavenging activity on DPPH

The DPPH scavenging test is a generally accepted means for assessing the scavenging activity of natural compounds on free radical [18]. The scavenging activity of PMP on DPPH radicals was determined, and the result was shown in Figure 3c. As Figure 3c showed at the concentration from 0.2 mg·mL^{-1} to 1.0 mg·mL^{-1}, the DPPH radical scavenging effect increased with the PMP concentration, but this increase became less obvious when the PMP concentration approached 1.0 mg·mL^{-1}, whereas the overall DPPH radical scavenging activity was not as strong as ascorbic acid. The results manifested that PMP had a noticeable effect on scavenging DPPH free radicals. This was in agreement with the earlier research on the antioxidant activity of various polysaccharides such as GSP [18], PSP [20].

CONCLUSIONS

In this study, the factors and levels of influence on PMP extraction process were first studied using the single factor investigation method, then the extraction conditions were optimized by RSM so as to maximize the yield of PMP, the relational expression between the factors levels and response value were established, and the mathematical

equation was evaluated with a statistical method. The optimal alkali extraction conditions combination predicted by obtained mathematical model was confirmed that alkali concentration was 0.87 mol·L^{-1}, liquid-solid ratio was 23.1:1 mL·g^{-1}, extraction time was 1.61 h, and extraction temperature was 77.7 °C. At this optimal extraction conditions combination, the PMP yield predicted by the obtained model was 36.12 mg·g^{-1}, meanwhile the practical PMP yield obtained from experiment was 38.27 mg·g^{-1}, and both values were fairly match. This explained that the mathematical model had higher correlation for experiment results and could be better applied to the extraction process condition optimization of PMP. The research of measuring antioxidant activity in vitro of PMP indicated that PMP had effective superoxide, hydroxyl and DPPH radical-scavenging activity.

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