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Optimization of acid assisted extraction process of foxtail millet polysaccharides and its antioxidant activity

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A b s t r a c t. Experiments concerning hydrochloric acid assisted extraction of polysaccharides from foxtail millet were carried out. Various combinations of extraction conditions of hydrochloric acid concentration, liquid-solid ratio, extraction time and extraction temperature were investigated using the Response Surface Methodology at three levels and four variables of Box-Behnken designs to obtain the maximum yield of polysaccharides. The experimental data were fitted to a quadratic equation using multiple regression analysis and analysed. The antioxidant activity of the obtained polysaccharides was also investigated *in vitro*. The optimal conditions were an acid concentration of 2.4 mol L^{-1}, a liquid-solid ratio of 19.3:1 mL g^{-1}, an extraction time of 1.2 h, and an extraction temperature 80.6°C. Under these conditions, the experimental yield was 52.13 mg g^{-1}, which was in close agreement with the value of 52.37 mg g^{-1} predicted by the model. The mathematical model had a high correlation with experimental data ($p<0.05$). The polysaccharides of foxtail millet display a high radical scavenging capacity against superoxide and hydroxyl radicals.

K e y w o r d s: foxtail millet, polysaccharides, acid extraction, response surface methodology, antioxidant activity

INTRODUCTION

Millet is an important crop for fodder and human food in the semiarid tropics of Asia and Africa, especially in India, Mali, Nigeria, China, and Niger. Foxtail millet is an important crop species of millet. It is rich in calcium, dietary fibre and polyphenols, which benefit human health (Radhika, *et al*., 2011). The research results show that foxtail millet also contains polysaccharides (Zhu, *et al*., 2014). In recent decades, numerous studies have demonstrated that polysaccharides have the therapeutic value of scavenging free radicals and few biological side effects; therefore, many researchers are paying more attention to polysaccharides as natural antioxidants (Guo *et al*., 2016; Wang *et al*., 2014a, b; Yuan *et al*., 2017). Response surface methodology (RSM) is widely used to optimize the extraction process variables, this is due to its efficiency at optimizing the complex processes variables (Quan *et al*., 2015; Osman *et al*., 2016; Tian *et al*., 2017; Zeng *et al*., 2015).

Almost no reports are available in the literature concerning studies to optimize the acid assisted extraction conditions of foxtail millet polysaccharides (FMP) and its antioxidant activity in vitro. The method of polysaccharide extraction using acidic aqueous solution can improve the purity, the yield and the activity of the polysaccharides extracted (Yaich *et al*., 2013). However, an excessively high acid concentration was not applied to improve the yield of polysaccharides, because overly acid solutions may accelerate the degradation of polysaccharides (Gan *et al*., 2010). Therefore, the optimization of processing conditions for the acid assisted extraction of FMP and its antioxidant activity in vitro were researched in this study. The effects of hydrochloric acid aqueous solution concentration (mol L^{-1}), liquid-solid ratio (solution volume versus material mass, mL g^{-1}), extraction time (h), and extraction temperature (°C) on the yield of FMP were studied respectively. Then

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RSM was employed to optimize the acid assisted extraction conditions and to roundly evaluate the degree of influence of the extraction parameters on the yield of FMP and the interactions between them. The radical scavenging activity against superoxide and the hydroxyl radical-scavenging activity of FMP were further studied *in vitro*.

MATERIALS AND METHODS

The product of foxtail millet was obtained from Jilin province Yuanfang agriculture development CO., LTD, Songyuan, China, 2017 of harvest. It was first dried at 60.0°C in a digital constant temperature drying oven (WX881-TG, Wujiang City Prestige Electric Equipment CO., LTD, Jiangsu, China) until a constant mass was obtained, then it was ground into powder with a masher (SY-1200-1, Guangzhou City Shanyou Machinery CO., LTD, Guangzhou, China). The mashed powder was sieved with 50-mesh and then packed into plastic bags for the extraction experiments. A digital electrically heated thermostatic water bath (HH-S4, Jintan City Jinnan Instrument Manufacturing CO., LTD, Jiangsu, China) was used to control the temperature of the experiments. A rotary evaporator (YRE-201D, Gongyi City Yuhua Instrument CO., LTD, Henan, China) was applied to concentrate the filtrate. A centrifuge (TGL-16G, Changzhou Meixiang Instrument CO., LTD, Jiangsu, China) was used to centrifuge the suspension solution obtained. The concentration of polysaccharides in the extracting solution was measured with a UV-Vis spectrophotometer (T6v, Nanjing Feile Instrument CO., LTD, Jiangsu, China). All chemical reagents were analytically pure.

The foxtail millet powder dosage of each experiment was 5.0 g. The raw material was placed into a three-neck flask (250 mL), and then it was extracted with hydrochloric acid solution without stirring. In the pre-experiment, it was found that the polysaccharide yield obtained from the second extraction was only approximately 1% of the first, so the extraction was carried out only once at different hydrochloric acid concentrations (2.0, 2.2, 2.4, 2.6 and 2.8 mol L^{-1}), liquid-solid ratios (10.0, 15.0, 20.0, 25.0 and 30.0 mL g^{-1}), extraction time (0.5, 1.0, 1.5, 2.0 and 2.5 h), extraction temperature (50, 60, 70, 80 and 90°C), respectively. After the extraction, the mash was first cooled with cold water, and then vacuum-filtered through a Buchner funnel. The obtained filtrate was placed in a rotary evaporator, and concentrated to one-fifth of the initial volume under vacuum. Four volumes of dehydrated ethanol were added to the concentrate obtained, and mixed to form a solution, which had a final ethanol concentration of 80% in order to alcohol-precipitate the polysaccharides. The suspension solution obtained was centrifuged at 2000 r.p.m. for 10 min, the supernatant fluid was removed and the precipitate was retained, after that the dehydrated ethanol was used to wash the precipitate three times. The precipitate was collected as product and dissolved with deionized water to reach a certain volume. Each extraction experiment was performed in triplicate.

In order to determine the best combination of extraction conditions for the yield of FMP, an experiment plan with three-levels and four-variables was designed through Box-Behnken factorial Design (BBD). In this research the four extraction variables were; hydrochloric acid concentration (X_1, mol L^{-1}), liquid-solid ratio (X_2, mL g^{-1}), extraction time (X_3, h), and extraction temperature (X_4, °C), and the appropriate investigated range of each variable was determined by referring to the single factor pre-experimental result. The FMP yield was used as the dependent variable. The whole experiment design consisted of 29 experimental points (including five replicated experiments in the centre point) and the experiment was put into practice in a random order. The obtained experimental data were fitted with a nonlinear quadratic equation Eq. (1):

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

where: Y is the response value, *i.e*. FMP yield obtained from each extraction experiment (mg g^{-1}); β_0, β_i, β_{ii}, β_{ij} are constants of the regression equation, dimensionless; X is the actual value of the independent variables; X_iX_j is the interaction between independent variables; and X_i^2 is the quadratic term of each independent variable (Lei *et al*., 2016; Zhu *et al*., 2017).

The FMP concentration was determined using the colorimetric method of phenol-sulphuric acid at 485 nm (Guo *et al*., 2016; Wang *et al*., 2014b). Eq. (2) below was used to calculate the yield of FMP (mg g^{-1}):

$$Y = \frac{XV}{M}, \quad (2)$$

where: Y is FMP yield (mg g^{-1}); V is the metered volume of the crude FMP solution (mL); M is the dosage of foxtail millet in each extraction experiment (g); X is the concentration of FMP in the extraction solution at 485 nm (mg mL^{-1}).

The scavenging of superoxide by antioxidants was estimated by the inhibition of pyrogallol acid as described previously (Wang *et al*., 2014a). In brief, 4.5 mL of 50 mM Tris-HCl buffer (pH 8.2) was mixed with 4.2 mL of deionized water. After incubation at 25°C for 20 min, 1 mL of FMP solution (0.2-1.0 mg mL^{-1}) and 0.4 mL of pyrogallic acid were added to the mixture. The resulting mixture was rapidly shaken and incubated at 25°C for 5 min. Subsequently, 8 mM of HCl was added to the mixture to terminate the reaction, and its absorbance was measured at wavelength 320 nm. Ascorbic acid was used as a positive control. The ability of FMP to scavenge superoxide radicals was calculated using the following formula:

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

where: A_0 is the absorbance of the blank and A_1 is the absorbance of FMP/Vc.

Hydroxyl radical scavenging activity was measured according to the procedures of Li *et al*. (2014). The reaction mixture (2.5 mL) contained 0.5 mL of $FeSO_4$ (1.5 mM), 0.35 mL of H_2O_2 (6.0 mM), 0.15 mL of sodium salicylate (20.0 mM), and 1.0 mL of different concentrations (0.2-1.0 mg mL^{-1}) of polysaccharides. Ascorbic acid was used as the positive control. After incubation for 60 min at 37°C, the absorbance of the hydroxylated salicylate complex formed was measured at a wavelength of 562 nm. The percentage scavenging effect was calculated according to Eq. (4) below:

$$\text{hydroxyl radical-scavenging activity}\,(\%) = \left(1 - \frac{A_1 - A_0}{A_2}\right) 100, \qquad (4)$$

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

Multiple non-linear regressions of the experimental data obtained were carried out using Design Expert Software. The coefficient of determination R^2 was used to express the quality of the fit of the polynomial model, and the significance of the regression coefficient was checked by the *F*-test and p-value. The significant difference was denoted by $p < 0.05$.

RESULTS AND DISCUSSION

In order to confirm the centre value of each independent variable in the response surface experiment, the pre-experiment of the single factor experiment was implemented before the optimization experiment. The hydrochloric acid concentrations were 2.0, 2.2, 2.4, 2.6 and 2.8 mol L^{-1}, the liquid-solid ratios were 10, 15, 20, 25 and 30 mL g^{-1}, the extraction times were 0.5, 1.0, 1.5, 2.0 and 2.5 h, the extraction temperatures were 50, 60, 70, 80 and 90°C. The single factor experimental result showed that the FMP yield reached a maximum value at 2.4 mol L^{-1} hydrochloric acid concentration, 20:1 mL g^{-1} liquid-solid ratio, 1.0 h extraction time, 80°C extraction temperature, respectively. Thus the range of 2.2-2.6 mol L^{-1} of hydrochloric acid concentration, 15.0-25.0 mL g^{-1} of liquid-solid ratio, 0.5-1.5 h of extraction time and an extraction temperature of 70-90°C (Table 1) were the conditions adopted in the RSM experiment.

The investigated factors and their levels, the experiment design plan and the result of each experiment are listed in Table 1. The response values of the FMP yield obtained from the research and the actual values of the parameters were fit to a second order polynomial equation (Eq. (5)), and a regression analysis was carried out. All of the analysis results are summarized in Table 2:

$$\begin{aligned} Y = {} & -928232 + 483113X_1 + 5.190X_2 + 93.550X_3 + 7.258X_4 + 0.065X_1X_2 \\ & - 2.650X_1X_3 + 0.010X_1X_4 - 0.079X_2X_3 + 0.009X_2X_4 - 0.157X_3X_4 \\ & - 100090X_1^2 - 0.154X_2^2 - 30.319X_3^2 - 0.045X_4^2 \end{aligned} \qquad (5)$$

when the value of probability (p) of some item is less than 0.05, this item has a significant effect on the FMP yield within the range of the investigation. The analysis of variance demonstrates that the regression equation obtained has a high significance level ($p < 0.0001$) with an *F* value of 36.43, and its suitability for use as experimental data is further evidenced by the values of the determination coefficient R^2 and the adjus ted R^2, which are 0.9733 and 0.9466 respectively. In order to examine the adequacy of the fitting model and confirm whether it would lead to bad or misleading results, a residual analysis was carried out. The residual and the effect diagrams of the experimental data are shown in Fig. 1 (Maran *et al*., 2013). As seen in Fig. 1a, the predicted values of the model are fairly close to the actual values of the experiments, all of the experimental and predicted points are near the 45º line, and this suggests that the regression equation obtained could successfully reveal the relationship between the variables and the dependent variables of the process. The normal % probability chart of residuals for dependent variables shown in Fig. 1b is normally distributed, because all of the points lie closely on a straight line and show no variance deviation. The degree of fit of the regression equation was analysed by drawing a diagram of the relationship between the internally studentized residuals and the predicted values, the results show that all of the points are located within the limit values (Fig. 1c). Since all of the leverage values are below 1 (Fig. 1d), there are no outliers or unexpected errors in the regression equation. At the same time, the difference in the beta values plot in Fig. 1e shows no excessive influence of observation on the regression coefficients. Fig. 1f shows that the Cook's distance values are located within the determined range, this reveals that there are no impact observations in the experimental data. Hence, no obvious patterns are found in the analysis of the regression equation and manifested in the accuracy of the obtained pattern. The results of the variance and residual analysis certify a good consistency of the data from the BBD experiment, and the FMP yield could be accurately predicted using the regression equation obtained. The p-value (<0.0001) of the model is less than 0.01, indicating that the regression equation (Eq. (5)) could give a better fit to the experimental data. The linear term of X_3 and each quadratic term ($p < 0.01$) also show an extremely significant effect on the yield of FMP. The linear term of X_2 and X_4 ($p < 0.05$) are significant. The linear term of X_1 and six interactive terms are nevertheless not significant ($p > 0.05$).

The relationship between the responses and the experimental variables may be illustrated graphically using three-dimensional (3D) response surface plots (Fig. 2) to

Table 1. Experimental design and results of alkali extraction

Test number	Acid concentration	Liquid-solid ratio	Extraction time	Extraction temperature	Y (mg g^{-1})
	X_1 (mol L^{-1})	X_2 (mL g^{-1})	X_3 (h)	X_4 (°C)	
1	2.2	15.0	1.0	80	45.07
2	2.6	15.0	1.0	80	45.38
3	2.2	25.0	1.0	80	43.03
4	2.6	25.0	1.0	80	43.60
5	2.4	20.0	0.5	70	32.71
6	2.4	20.0	1.5	70	45.78
7	2.4	20.0	0.5	90	35.86
8	2.4	20.0	1.5	90	45.79
9	2.2	20.0	1.0	70	39.59
10	2.6	20.0	1.0	70	40.31
11	2.2	20.0	1.0	90	42.46
12	2.6	20.0	1.0	90	43.26
13	2.4	15.0	0.5	80	33.40
14	2.4	25.0	0.5	80	31.60
15	2.4	15.0	1.5	80	45.77
16	2.4	25.0	1.5	80	43.18
17	2.2	20.0	0.5	80	31.56
18	2.6	20.0	0.5	80	33.45
19	2.2	20.0	1.5	80	45.85
20	2.6	20.0	1.5	80	46.68
21	2.4	15.0	1.0	70	43.45
22	2.4	25.0	1.0	70	40.69
23	2.4	15.0	1.0	90	43.71
24	2.4	25.0	1.0	90	42.65
25	2.4	20.0	1.0	80	50.20
26	2.4	20.0	1.0	80	51.74
27	2.4	20.0	1.0	80	51.79
28	2.4	20.0	1.0	80	50.38
29	2.4	20.0	1.0	80	50.88

investigate the interactions of the variables. Each plot showed a pair of factors by keeping the other factors constant at its middle level.

The 3D response surface plot for extraction yield as a function of acid concentration and liquid-solid ratio at a fixed extraction time (1.0 h) and extraction temperature (80°C) is given in Fig. 2a. It may be seen that the contributions of acid concentration and liquid-solid ratio to the tendency and strength of the effect on the yield are similar, there is an increase followed by a decrease in yield with an increase in acid concentration and liquid-solid ratio. Acid can help to weaken the chemical and physical effect between the polymer molecules of the cell wall causing more polysaccharides to dissolve from the cells into solution, for this reason the FMP yield is increased, whereas an excessively high acid concentration would bring about a reduction in the FMP yield as a result of polysaccharide structure breakdown caused by acid catalysed hydrolysis (Bendahou *et al*., 2007). The extraction yield increased at first with the increase in the liquid-solid ratio due to the driving force rising for the mass transfer with the augmentation of the liquid-solid ratio (Guo *et al*., 2016). However, the yield clearly decreases with the continuously increasing ratio, because of the acid-catalysed hydrolysis. These results are similar to those of earlier observations (Guo *et al*., 2016; Wang *et al*., 2014b; Yuan *et al*., 2017; Zeng *et al*., 2015). Fig. 2b shows the 3D response of the surface plot with varying acid concentration and extraction time under a liquid-solid ratio of 20.0 mL g^{-1}, and an extraction temperature of 80°C. It demonstrates that the extraction time

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

Source	Sum of squares	df	Mean square	F value	Prob > F	Significance
Model	975.15	14	69.65	36.43	<0.0001	‡
X_1	2.18	1	2.18	1.14	0.3032	*
X_2	12.06	1	12.06	6.31	0.0249	†
X_3	462.15	1	462.15	241.74	<0.0001	‡
X_4	10.45	1	10.45	5.47	0.0347	†
X_1X_2	0.017	1	0.017	8.840×10^{-3}	0.9264	*
X_1X_3	0.28	1	0.28	0.15	0.7072	*
X_1X_4	1.600×10^{-3}	1	1.600×10^{-3}	8.369×10^{-3}	0.9773	*
X_2X_3	0.16	1	0.16	0.082	0.7793	*
X_2X_4	0.72	1	0.72	0.38	0.5486	*
X_3X_4	2.46	1	2.46	1.29	0.2752	*
X_1^2	103.97	1	103.97	54.38	<0.0001	‡
X_2^2	95.76	1	95.76	50.09	<0.0001	‡
X_3^2	372.67	1	372.67	194.94	<0.0001	‡
X_4^2	131.41	1	131.41	68.74	<0.0001	‡
Residual	26.76	14	1.91			
Lack of fit	24.55	10	2.46	4.44	0.0818	*
Pure error	2.21	4	0.55			
Cor total	1001.91	28				
		$R^2 = 0.9733$		Adj. $R^2 = 0.9466$		

*Not significant, † significant, ‡ extremely significant, df – degrees of freedom.

has a similar effect on yield as the liquid-solid ratio, but the influence of the extraction time is greater than that of the acid concentration. This is possible, because the process of breaking the foxtail millet cell wall and the penetration of liquid into the dried powdered foxtail millet causing the dissolution of the polysaccharides, which subsequently diffuse out from the material to the exterior solvent, requires a certain time (Guo *et al*., 2016). However, the FMP yield is reduced with increasing extraction time. It is supposed that polysaccharides dissolved in acid solution were hydrolysed because of their long period of time in solution (Chen *et al*., 2007). This result is in agreement with the observations of Wang *et al*. (2014b), Yuan *et al*. (2017), and Guo *et al*. (2016). The 3D response surface plot for the extraction yield as a function of acid concentration and extraction temperature at a fixed liquid-solid ratio (20.0 mL g^{-1}) and extraction time (1.0 h) is given in Fig. 2c. From Fig. 2c we may observe that the extraction yield is increased first with the increase in the extraction temperature, and then the extraction yield is decreased after the peak. With the increase in temperature, the solvent viscosity is decreased to improve the solvent and solute diffusivity in the suspension solution system, which improves the solubility capacity of the polysaccharides (Wang *et al*., 2014b). However, a high temperature would cause the yield to decrease due to the degradation of polysaccharides at a high temperature (Ren *et al*., 2008). This result is consistent with the studies concerning polysaccharides from *Monascus mycelium* by Wang *et al*. (2014b) and the polysaccharides from Huaguoshan Yunwu tea by Yuan *et al*. (2017). The 3D response surface plot at varying liquid-solid ratios and extraction times in a fixed acid concentration of 2.4 mol L^{-1}, extraction temperature 80°C; the liquid-solid ratio and extraction temperature were fixed at an acid concentration of 2.4 mol L^{-1}, extraction time 1.0 h; extraction time and extraction temperature at a fixed acid concentration of 2.4 mol L^{-1}, and a liquid-solid ratio of 20.0 mL g^{-1} is given in Fig. 2d, Fig. 2e and Fig. 2f, respectively. Fig. 2d shows the changed trend of the yield with increases in extraction time and liquid-solid ratio. Fig. 2e shows the changed trend of the yield with increases in extraction temperature and liquid-solid ratio, and Fig. 2f shows the changed trend of yield with increases in extraction time and extraction temperature. Their changed trends are similar, *i.e.* at first the yield is increased and then decreased with the increase in each factor, moreover the influence of extraction time is similarly greater than the liquid-solid ratio or extraction temperature.

It may be determined from Eq. (5) that the best extraction conditions of FMP are 2.41 mol L^{-1} of acid concentration, 19.31:1 mL g^{-1} of liquid-solid ratio, 1.20 h extraction time, 80.62°C extraction temperature, and the highest FMP yield as predicted by Eq. (5) is 52.37 mg g^{-1} for this combination of conditions.

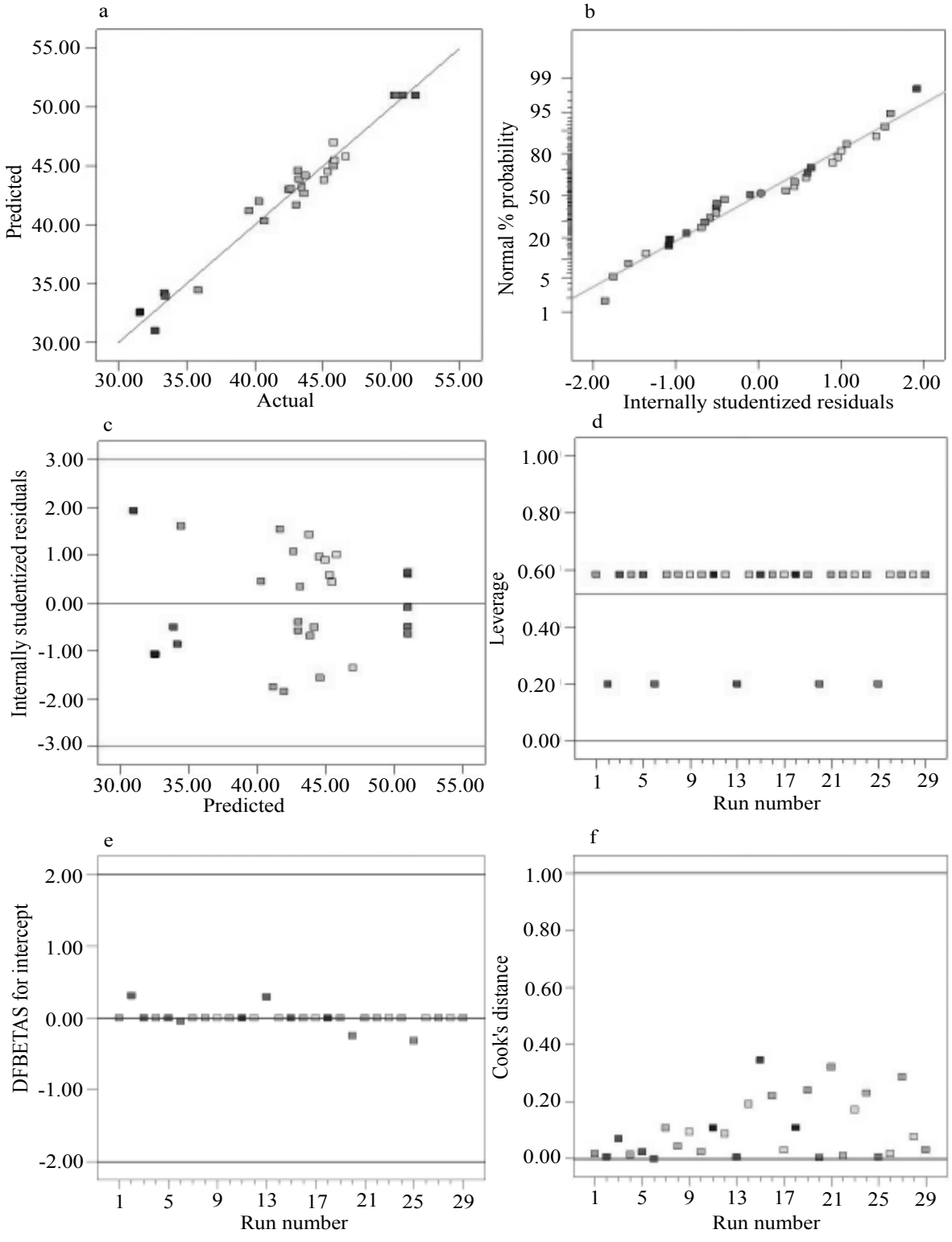
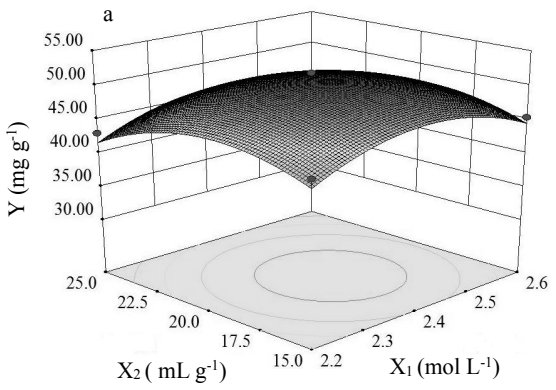
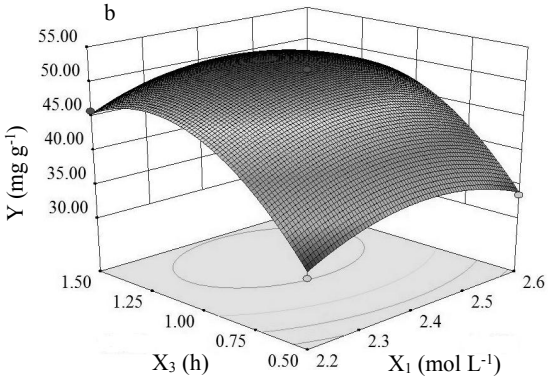


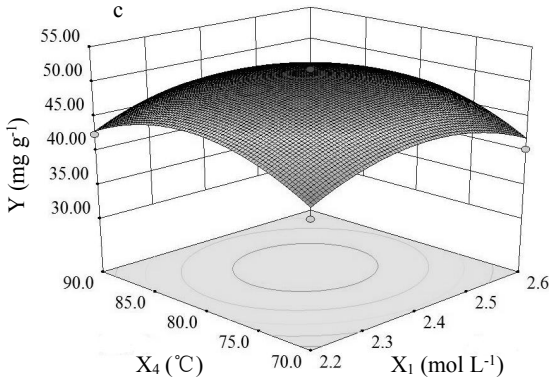
Fig. 1. Diagnostic plots for the model adequacy of acid extraction.



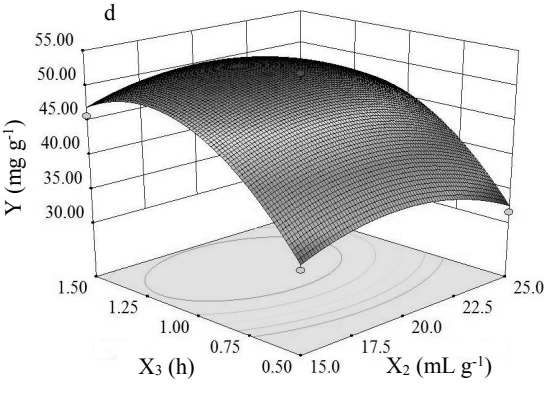
a. Response surface plot of Y=f(X_1, X_2)
(X_3=1.0 h, X_4=80.0 ℃)



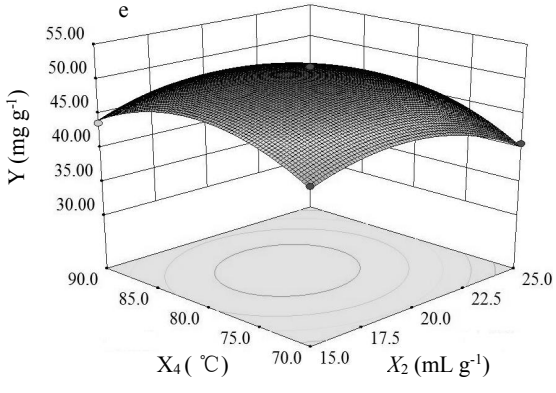
b. Response surface plot of Y=f(X_1, X_3)
(X_2=20.0 mL g^{-1}, X_4=80.0 ℃)



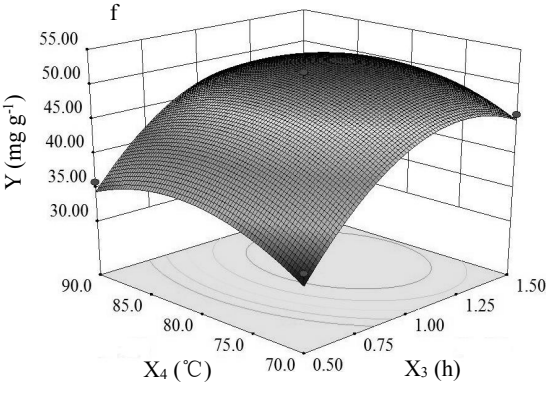
c. Response surface plot of Y=f(X_1, X_4)
(X_2=20.0 mL g^{-1}, X_3=1.0 h)



d . Response surface plot of Y=f(X_2, X_3)
(X_1=2.4 mol L^{-1}, X_4=80.0 ℃)



e. Response surface plot of Y=f(X_2, X_4)
(X_1=2.4 mol L^{-1}, X_3=1.0 h)



f. Response surface plot of Y=f(X_3, X_4)
(X_1=2.4 mol L^{-1}, X_2=20.0 mL g^{-1})

Fig. 2. Response surface plots of the effects of different combinations of conditions on polysaccharides yield.

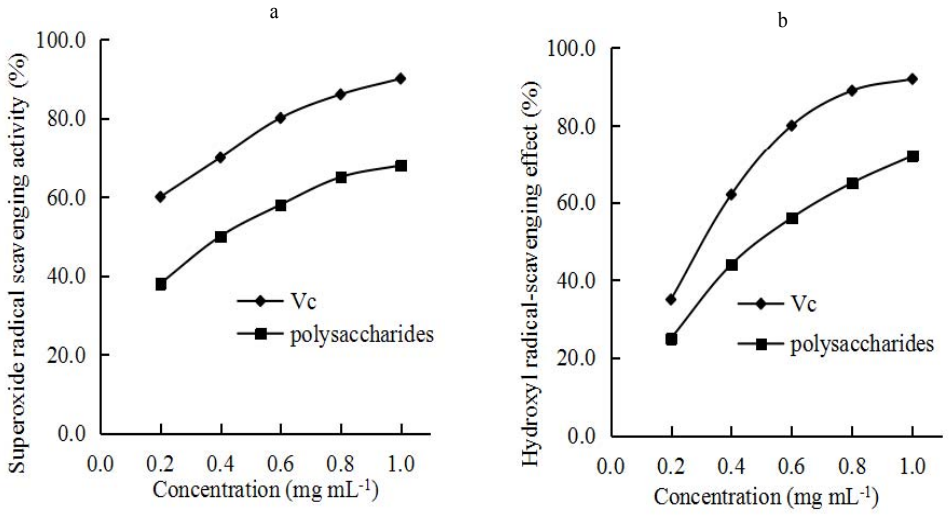


Fig. 3. Radical-scavenging activities (%) of the polysaccharides and ascorbic acid on (a) superoxide anion and (b) hydroxyl.

The parallel tests were implemented three times to determine the accuracy of the prediction of Eq. (5) at an acid concentration of 2.4 mol L^{-1}, a liquid-solid ratio of 19.3:1 mL g^{-1}, an extraction time of 1.2 h, and an extraction temperature of 80.6°C. The average experimental value of the FMP yield was 52.13±0.73 mg g^{-1} (n=3), which is lower by 0.46% as compared with the value of 52.37 mg g^{-1} predicted by Eq. (5). This result could ultimately prove that the mathematical model obtained could be adequately used to predict optimization, and that the accuracy of Eq. (5) is satisfactory.

The superoxide radical, one of the precursors of singlet oxygen and hydroxyl radicals, indirectly initiates lipid peroxidation. The presence of superoxide anions can also aggravate cellular damage because of their ability to produce other kinds of free radicals and oxidizing agents (Wang *et al*., 2014a). The scavenging activities of FMP on superoxide anion are presented in Fig. 3a. It is obvious that the scavenging ability of FMP on superoxide radicals correlated positively with increasing concentrations in the range of the investigated concentration, however, this increase became less obvious when the concentration approached 1.0 mg mL^{-1}. The results demonstrated that FMP had a noticeable effect on inhibiting the formation of superoxide radicals especially at high concentrations, although the superoxide radical scavenging rate of FMP was lower than that of ascorbic acid. Similar results have been reported for other plant polysaccharides (Wang *et al*., 2014a; Zhao *et al*., 2013).

It is important to remove hydroxyl radicals through antioxidant defence because hydroxyl radicals are one of the reactive oxygen species generated in the body, which can easily cross cell membranes, rapidly react with most biomolecules, and inflict tissue damage or cell death (Li *et al*., 2014; Zhao *et al*., 2013). As shown in Fig. 3b, FMP exhibited a concentration-dependent hydroxyl radical scavenging activity in the range of the investigated concentration. The results indicated that FMP had a high level of hydroxyl radical scavenging effect, but the scavenging ability of FMP on hydroxyl radicals is lower than that of the ascorbic acid. The results were generally in agreement with some of the literature concerning the study of various polysaccharides antioxidant activities (Zhang *et al*., 2014; Zhao *et al*., 2013).

CONCLUSIONS

1. The research found that the liquid-solid ratio, extraction time and extraction temperature had a significant effect on the extraction yield of foxtail millet polysaccharides through the analysis of response surface methodology.

2. The obtained quadratic equation had a high correlation with the experimental data, and could be effectively used to optimize the extraction process of foxtail millet polysaccharides. It was found that the optimal extraction conditions were a combination of 2.4 mol L^{-1} acid concentration, 19.3:1 mL g^{-1} liquid-solid ratio, 1.2 h extraction time and 80.6°C extraction temperature. At the optimal combination of conditions, the foxtail millet polysaccharides yield was predicted through the use of a mathematical model to be 52.37 mg g^{-1}, and the real yield obtained from experiment was 52.13 mg g^{-1} under the same optimal conditions, both the predicted and the experimental yield values were essentially in agreement.

3. The polysaccharides were found to have significant superoxide and hydroxyl radical-scavenging activity.

Conflict of interests: The authors declare that they have no conflict of interest.

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