CI&CEQ
Chemical Industry & Chemical Engineering Quarterly

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Chem. Ind. Chem. Eng. Q. 23 (1) 141–150 (2017) CI&CEQ

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SCIENTIFIC PAPER

UDC 633.17:577.114.4:66.061:66

https://doi.org/10.2298/CICEQ151011026Z

COMPARING OF HOT WATER AND ACID EXTRACTION OF POLYSACCHARIDES FROM PROSO MILLET

Article Highlights

- Polysaccharides were extracted from proso millet with hot water and acid solution
- Response surface methodology was used and a model was set up
- The best extraction methods were obtained
- The polysaccharides yield of acid extraction was significantly higher than hot water extraction

Abstract

The extraction of polysaccharides from proso millet was investigated experimentally using hot water and acid aqueous solution. Response surface methodology, based on a three-level, three- or four-variable Box-Behnken design for hot water extraction or acid extraction, respectively, was employed to obtain the best possible combination of acid concentration, liquid-solid ratio, extraction time, and extraction temperature for maximum polysaccharides yield. The obtained experimental data were fitted to a second-order polynomial equation and analyzed by appropriate statistical methods. The corresponding optimum extraction conditions of each method were obtained. Under the optimum conditions, the experimental yield was well in close agreement with the predicted value by the model. The results showed that the polysaccharides yield of acid extraction was 42.13 mg g^{-1}, significantly higher than 20.07 mg g^{-1} of the yield of hot water extraction, the obtained equation could be used to predict the extraction experimental results.

Keywords: proso millet, polysaccharides, hot water extraction, acid extraction, response surface methodology.

The proso millet (*Panicum miliaceum* L.) is a warm-season grass with a short growing season and low moisture requirement that is capable of producing food or feed where other grain crops would fail. It is the a widely planted species of millet; is extensively cultivated for its grain in the arid areas of China, India, Russia, Ukraine, the Middle East, Turkey and Romania. The seeds as grain are small (2–3 mm) and rich in starch, protein, fat, dietary fiber, vitamin and trace elements. It has many functions beneficial to human health, such as the prevention and management of diabetes mellitus [1], callus and shoots regeneration from protoplasts of proso millet [2]. Polysaccharides have unique biological properties such as anti-oxidative [3], anti-viral [4] and anti-complementary activities [5], as well as chemical and physical properties [6]. They have important effects in the process of growth and development for living organisms [7].

Response surface methodology (RSM) is an effective statistical technique for optimizing complex processes. It is widely used in optimizing the extraction process variables [8,9].

Kim *et al.* [1] studied the inhibitory effects of ethanol extracts from proso millet on α-glucosidase and α-amylase activities, Heyser [2] studied the callus and shoot regeneration from protoplasts of proso millet, Yañez *et al.* [10] studied some chemical and physical properties of proso millet starch. However, there is no report about polysaccharides extraction from proso millet, especially extraction with acid sol-

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Paper received: 11 October, 2015
Paper revised: 2 January, 2016
Paper accepted: 26 April, 2016

ution. In this article, we report on the extraction of polysaccharides from proso millet by hot water and acid solution. Polysaccharides extracted with the acidic aqueous solution can be made for a pure polysaccharide, greatly improve the yield of polysaccharide, and the activity of polysaccharide is higher [11], high acid concentration can accelerate the degradation of polysaccharides [8]. Based on the results of the single factor investigation, once extracted and stirring rate of 150 rpm in the extraction process, RSM was designed to help to optimize extraction variables of acid concentration (hydrochloric acid aqueous solution, mol L^{-1}), liquid-solid ratio (water or acid aqueous solution volume with proso millet mass, mL g^{-1}), extraction time (h) and extraction temperature (°C) and to systematically analyzed the influence of the variables.

MATERIALS AND METHODS

Materials and extraction of polysaccharides

The decorticated grain proso millet was obtained from Donghua rice industry limited company, Jinzhou, Liaoning, China, 2014 harvest. The size of proso millet particle was about 2–3 mm. Before the extraction experiment, the sample was placed first in electrothermal blast oven (DHG-9123A, Precision Experimental Facilities Limited Company, Shanghai, China) to dry at 60.0 °C to constant mass. Five grams of dry proso millet, weighed with an electronic balance (BS124S Sartorius Instrument System Limited Company, Beijing, China) and mixed with different volume of water or different concentration acid solution was extracted in a 500-ml three-neck flask. The three--neck flask was soaked in a thermostatic water bath (DK-S24 Jing Hong Experimental Facilities Limited Company, Shanghai, China) which controlled the needed extraction temperature, with stirring at 150 rpm with an electric mixing paddle for a given time during the entire extraction process. After extraction, the mash was quickly put in cold bath, cooled down to room temperature and then vacuum-filtered. The filtrate was concentrated in a rotary evaporator (RE52CS Yarong Biochemistry Instrument Plant, Shanghai, China) to 20% of the initial volume at 60.0 °C under vacuum. The obtained solution was mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) to obtain the precipitate. Then, the suspension was centrifuged using centrifuge (800B Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 4000 rpm for 15 min, the precipitate was collected as extract and washed three times with dehydrated ethanol. The extract was dried at 50 °C in electrothermal blast oven until constant mass and weighed. The concentration of polysaccharides content in the extract was examined with a spectrophotometer (722E Shanghai Spectrum Instruments Co., LTD, Shanghai, China). The proso millet was extracted once and the extraction procedure was repeated twice. All reagents were of analytical grade.

Experimental design

The Box-Behnken Design was applied to determine the best combination of extraction variables for the yield of proso millet polysaccharides. A three-level, three-variable experimental design was carried out to hot water extraction, and three extraction variables considered for this research were: liquid-solid ratio (mL g^{-1}, X_1), extraction time (h, X_2), and extraction temperature (°C, X_3). A three-level, four-variable experimental design was used to acid extraction, and four extraction variables considered for these researches were: acid 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) [8]. The reasonable range of variables was obtained through the single factor investigation. The polysaccharides extraction yield was as the dependent variable. The complete experiment scheme was consists of 17 or 29 experimental points (including five replicates of the center point) for three-level, three-variable experimental design or three-level, four-variable experimental design, respectively. A quadratic polynomial model was used to fit the obtained experimental data and the regression coefficients were obtained through statistical analyses. The mathematic expression of the quadratic polynomial model was seen Eq. (1) [8,12--14]:

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

where Y is the measured response (polysaccharides yield) of each experiment; β_0, β_i, β_{ii}, β_{ij} are constant regression coefficients of the model; X is the code levels of independent variables; X_iX_j and X_i^2 represent the interaction and quadratic terms of independent variables, respectively.

Analysis of samples

The phenol-sulphuric acid colorimetric method at 485 nm was used to determine the polysaccharides content [12,13]. In this method the glucose was used as standard, the polysaccharides yield (mg g^{-1}) was calculated using Eq. (2):

$$Y = \frac{1000XV}{M} \quad (2)$$

where Y is yield of polysaccharides, mg g^{-1}; V is the total volume of the coarse polysaccharides dissolved after the constant volume, L; M is dosage of raw material millet, g; X is concentration of polysaccharides in solution, mg L^{-1}. X was calculated using Eq. (3):

$$X = 0.0255A - 0.00006 \quad (3)$$

where A is the absorbance of measured solution at 485 nm.

Statistical analyses

The Design Expert Software (version 8.0.5.0, Stat-Ease Inc., Minneapolis, MN) was used to multiple nonlinear regressions of the responses obtained from each design experimental. 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 of the regression coefficient. p-Values below 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Hot water extraction

Model fitting. The factors and their levels of hot water extraction were chosen on the basis of single-factor experiments (Table 1). The experiment design and the yields are shown in Table 1. The regression analysis results are shown in Table 2.

Table 1. Experimental design and results of hot water extraction

Test number	Liquid-solid ratio	Extraction time	Extraction temperature	Y / mg g^{-1}
	X_1 / mL g^{-1}	X_2 / h	X_3 / °C	
1	20:1	1.0	65	7.85±0.22
2	20:1	1.0	75	16.11±0.33
3	20:1	2.0	65	6.20±0.29
4	20:1	2.0	75	17.91±0.33
5	15:1	1.5	65	4.14±0.21
6	15:1	1.5	75	17.19±0.25
7	25:1	1.5	65	7.63±0.27
8	25:1	1.5	75	15.24±0.29
9	15:1	1.0	70	16.52±0.34
10	15:1	2.0	70	14.88±0.31
11	25:1	1.0	70	14.75±0.27
12	25:1	2.0	70	17.94±0.21
13	20:1	1.5	70	19.82±0.22
14	20:1	1.5	70	19.20±0.22
15	20:1	1.5	70	19.46±0.22
16	20:1	1.5	70	19.34±0.22
17	20:1	1.5	70	19.27±0.22

*Table 2. Analysis of variance for regression equation to hot water extraction; R^2 = 0.9987, Adj. R^2 = 0.9970; **extremely significant; *significant*

Source	Sum of squares	*df*	Mean square	*F*-value	Prob > *F*	Significance
Model	423.54	9	47.06	597.64	< 0.0001	**
X_1	1.00	1	1.00	12.71	0.0092	**
X_2	0.36	1	0.36	4.59	0.0694	-
X_3	206.35	1	206.35	2620.53	< 0.0001	**
X_1X_2	5.83	1	5.83	74.07	< 0.0001	**
X_1X_3	7.40	1	7.40	93.96	< 0.0001	**
X_2X_3	2.98	1	2.98	37.79	0.0005	**
X_1^2	20.04	1	20.04	254.47	< 0.0001	**
X_2^2	6.21	1	6.21	78.81	< 0.0001	**
X_3^2	161.15	1	161.15	2046.50	< 0.0001	**

Table 2. Continued

Source	Sum of squares	*df*	Mean square	*F*-value	Prob > *F*	Significance
Residual	0.55	7	0.079			-
Lack of fit	0.31	3	0.10	1.74	0.2962	-
Pure error	0.24	4	0.060			-
Cor. total	424.09	16				-

The response surface plots graphs are shown in Figure 1. The response value Y (*i.e.*, the proso millet polysaccharides yield) can be expressed by Eq. (4) in terms of actual values:

$$Y = -1337.566 + 6.645X_1 - 18.817X_2 + 36.231X_3 + \\ +0.483X_1X_2 - 0.054X_1X_3 + 0.345X_2X_3 - \\ -0.087X_1^2 - 4.856X_2^2 - 0.247X_3^2 \quad (4)$$

The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized in Table 2. The value of probability (p) was less than 0.05, which indicates that the selected factors and their ranges have significant influence on the yield of polysaccharides. The residual analysis was then performed to check the adequacy of the developed model and determine whether the approximating model would give poor or misleading results. Figure 2 shows the residual and the influence plots for the experimental data [9]. The predicted values obtained are quite close to the experimental values, and the points of all predicted and experimental response values fall very close to the 45° line (Figure 2a), indicating that the model developed is successful in capturing the correlation between the process variables on the response. Figure 2b shows the normal probability plot of residuals for response is normally distributed, as they lie reasonably close on a straight line and shows no deviation of the variance. The goodness of fit of the model is analyzed by constructing the internally studentized residuals versus experimental runs and shows that all the data points lay within the limits (Figure 2c). Since the Cook's distance values are in the determined range (Figure 2d), there is no strong evidence of influential observations in experimental data. The above results indicate a good adequate agreement between BBD experimental data and the model could be better predicted yield of polysaccharides. Linear term of X_3 (extraction temperature, $p < 0.0001$) showed the largest effect on polysaccharide yield, followed by linear term of X_1 (liquid-solid ratio, $p = 0.0092 < 0.05$) , all interaction terms and all quadratic terms ($p < 0.0001$) were also extremely significant. Linear term of X_2 (extraction time, $p = 0.0694$) was however not significant ($p > 0.05$). The p-value of model was less than 0.0001 and Adj R^2 was 0.9970 which would give a better fit to the mathematical model (Eq. (4)).

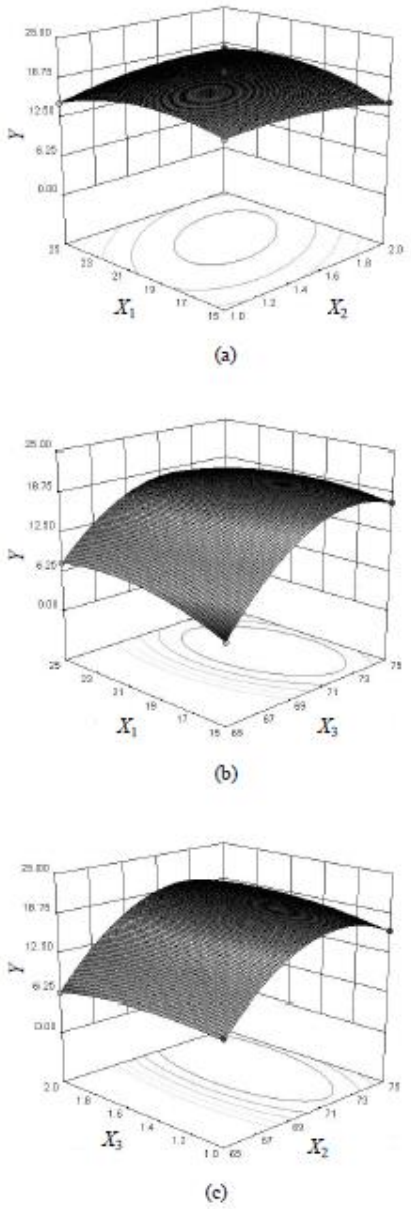

Figure 1. a) Response surface plot of Y = f(X_1,X_2) (X_3 = 70.0 °C); b) response surface plot of Y = f(X_1,X_3) (X_2 = 1.5 h); c) Response surface plot of Y = f(X_2,X_3) (X_1 = 20.0 mL g^{-1}); X_1: liquid-solid ratio (mL g^{-1}), X_2: extraction time (h), X_3: extraction temperature (°C), Y: yield (mg g^{-1}).

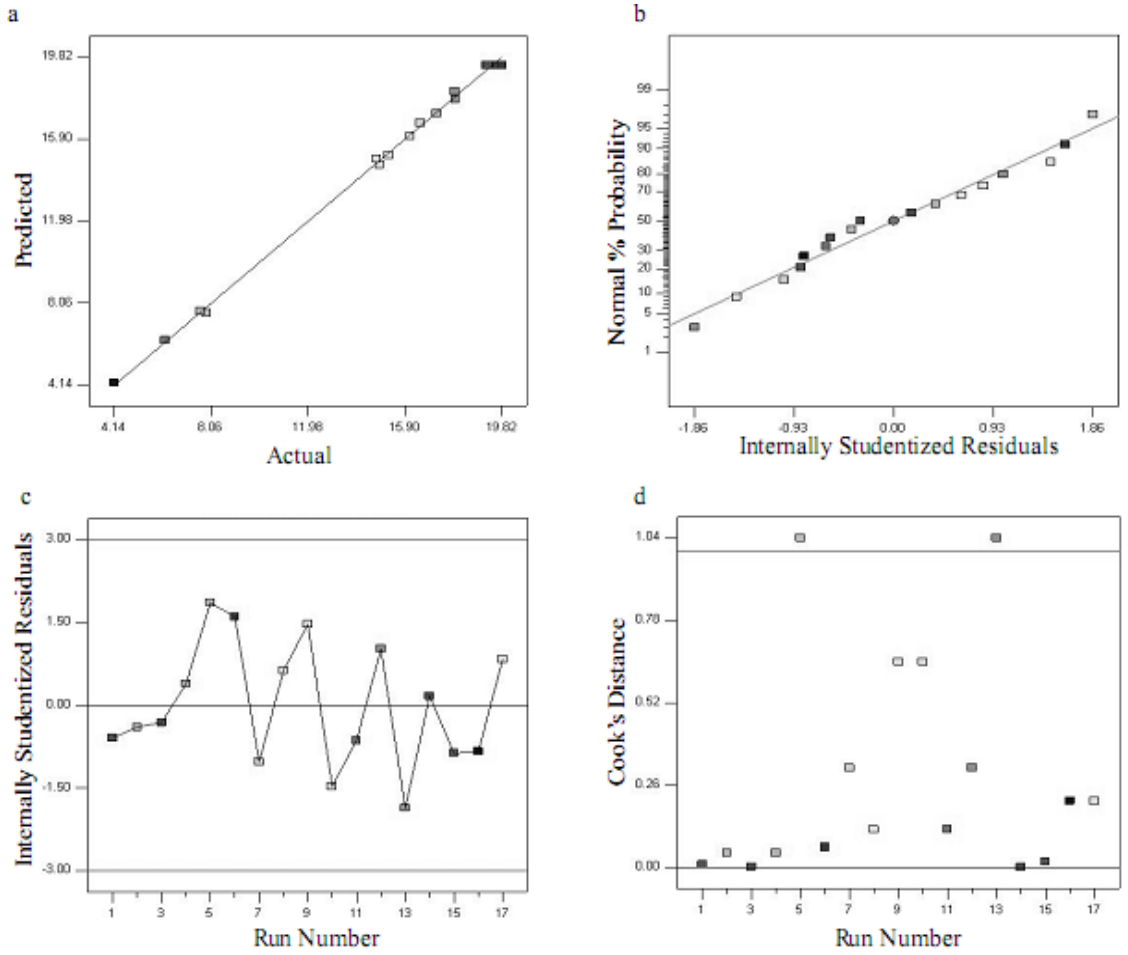

Figure 2. Diagnostic plots for the model adequacy of hot water extraction; a) predicted vs. actual, b) normal plot of residuals, c) residuals vs. run and d) Cook's distance.

Verification of the model. The regression model predicted the optimum extraction conditions of proso millet polysaccharides, which were liquid-solid ratio 19.77:1 mL g^{-1}, extraction time 1.85 h, extraction temperature 71.79 °C. Under this optimum extraction conditions, the polysaccharides yield was predicted for 20.17 mg g^{-1}.

The experiment was repeatedly carried out three times for verifying the prediction from the model at liquid-solid ratio 19.8:1 mL g^{-1}, extraction time 1.8 h and extraction temperature 71.8 °C. The average value of practical polysaccharides yield was 20.07± ±0.21 mg g^{-1}(n = 3), the relative error was -0.5% compared with predicted yield of 20.17 mg g^{-1}. The analysis results proved that the quadratic polynomial model was suitable for expressing the optimization results and the satisfaction and accuracy of Eq. (4) was high.

Acid extraction

Model fitting. The factors and their levels of acid extraction were chosen on the basis of single-factor experiments (Table 3). The experiment design proposal and the yields are shown in Table 3. The regression analysis results are shown in Table 4.

Table 3. Experimental design and results of acid 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	3.0	20:1	1.5	70	30.48±0.53
2	2.5	25:1	1.5	60	12.08±0.26
3	2.0	25:1	1.5	70	22.67±0.35
4	2.5	25:1	1.0	70	22.94±0.45

Table 3. Continued

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	
5	3.0	20:1	2.0	70	26.86±0.58
6	2.5	20:1	1.0	80	31.00±0.54
7	2.5	15:1	2.0	70	25.64±0.42
8	2.5	15:1	1.5	60	12.88±0.26
9	2.0	20:1	1.5	60	10.24±0.36
10	2.0	20:1	1.0	70	17.88±0.28
11	3.0	20:1	1.5	60	26.87±0.37
12	3.0	20:1	1.5	80	31.88±0.48
13	2.0	20:1	1.5	80	35.50±0.63
14	2.5	25:1	2.0	70	27.50±0.53
15	2.5	25:1	1.5	80	34.99±0.57
16	2.5	20:1	2.0	80	32.09±0.42
17	2.5	15:1	1.0	70	25.58±0.34
18	2.0	20:1	2.0	70	29.97±0.38
19	2.5	20:1	2.0	60	14.98±0.29
20	3.0	25:1	1.5	70	30.48±0.39
21	3.0	15:1	1.5	70	27.27±0.47
22	2.5	15:1	1.5	80	31.61±0.50
23	2.0	15:1	1.5	70	23.55±0.41
24	2.5	20:1	1.0	60	15.39±0.45
25	2.5	20:1	1.5	70	40.67±1.16
26	2.5	20:1	1.5	70	37.59±1.16
27	2.5	20:1	1.5	70	38.25±1.16
28	2.5	20:1	1.5	70	38.52±1.16
29	2.5	20:1	1.5	70	40.07±1.16

*Table 4. Analysis of variance for regression equation to acid extraction; R^2 = 0.9641, Adj. R^2 = 0.9281; **extremely significant; *significant*

Source	Sum of squares	*df*	Mean square	*F*-value	Prob > *F*	Significance
Model	2011.80	14	143.70	26.82	<0.0001	**
X_1	77.36	1	77.36	14.44	0.0020	**
X_2	0.099	1	0.099	0.019	0.8936	-
X_3	17.07	1	17.07	3.19	0.0960	-
X_4	913.18	1	913.18	170.42	<0.0001	**
X_1X_2	4.20	1	4.20	0.78	0.3911	-
X_1X_3	37.06	1	37.06	6.92	0.0198	*
X_1X_4	102.48	1	102.48	19.12	0.0006	**
X_2X_3	14.06	1	14.06	2.62	0.1276	-
X_2 X_4	4.44	1	4.44	0.83	0.3782	-
X_3 X_4	0.56	1	0.56	0.11	0.7504	-
X_1^2	213.43	1	213.43	39.83	<0.0001	**
X_2^2	316.17	1	316.17	59.00	<0.0001	**
X_3^2	320.45	1	320.45	59.80	<0.0001	**
X_4^2	448.60	1	448.60	83.72	<0.0001	**
Residual	75.02	14	5.36	-	-	-
Lack of fit	68.32	10	6.83	4.08	0.0938	-
Pure error	6.70	4	1.67	-	-	-
Cor. total	2086.82	28	-	-	-	-

The response surface plots are shown in Figure 3. The response value Y can be expressed by the following second order polynomial equation in terms of actual values:

$$Y = -906.997 + 200.730X_1 + 7.565X_2 + \\ +96.915X_3 + 14.512X_4 + 0.410X_1X_2 - \\ -12.175X_1X_3 - 1.012X_1X_4 + 0.750X_2X_3 + \\ +0.021X_2X_4 + 0.075X_3X_4 - 22.944X_1^2 - \\ -0.279X_2^2 - 28.115X_3^2 - 0.083X_4^2 \quad (5)$$

The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized in Table 4. Figure 4 shows the residual and the influence plots for the experimental data, it is similar to Figure 2. The linear term of X_1 (acid concentration, $p = 0.002$) and X_4 (extraction temperature, $p < 0.0001$) had an extremely significant effect on polysaccharide yield. Each quadratic term ($p <$ < 0.0001) and the interaction term of X_1 and X_4 ($p =$ $= 0.0006$) were also extremely significant. The inter-

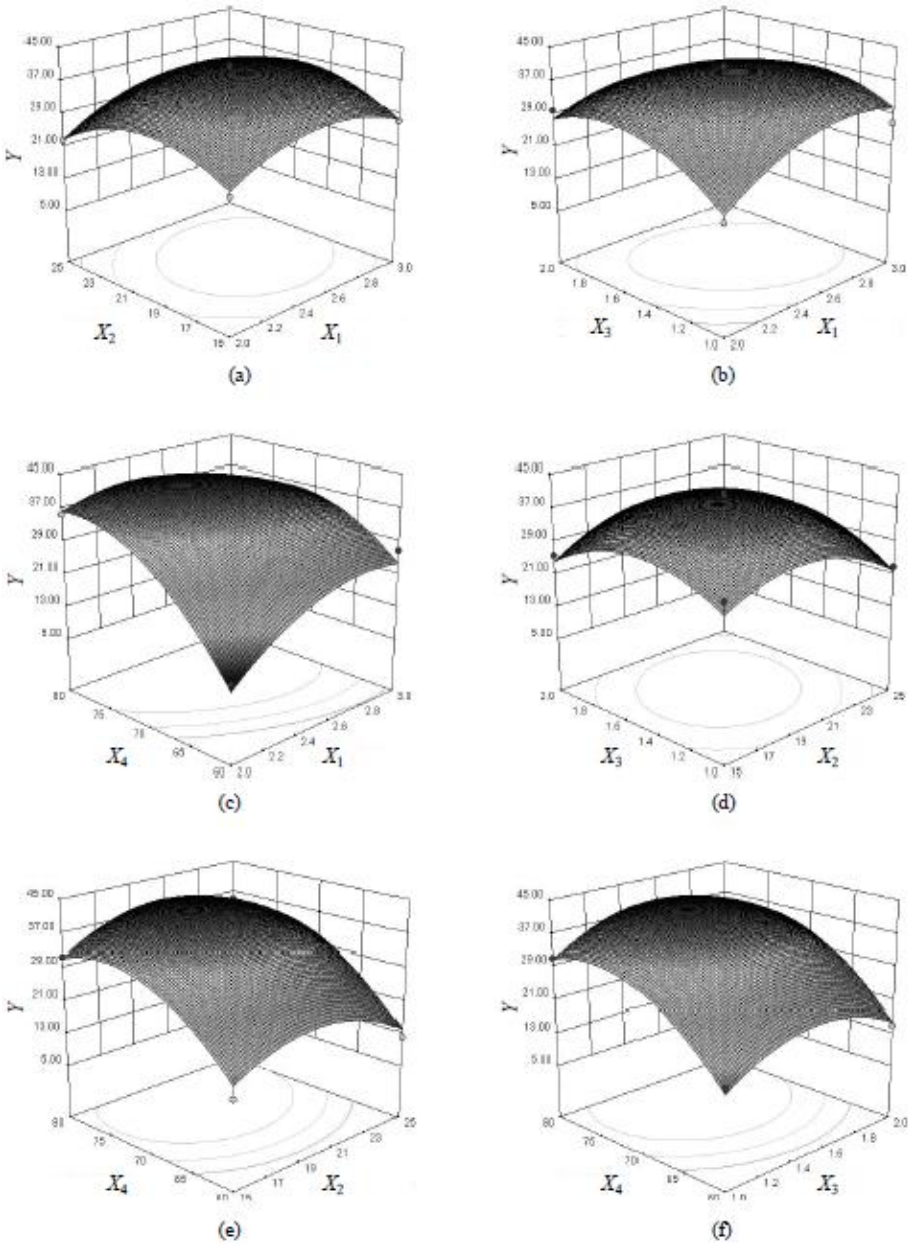

Figure 3. Response surface plots of: a) Y = f(X₁,X₂) (X₃ = 1.5 h, X₄ = 70.0 °C); b) Y = f(X₁,X₃) (X₂ = 20 mL g⁻¹, X₄ = 70.0 °C); c) Y = f(X₁,X₄) (X₂ = 20 mL g⁻¹, X₃ = 1.5 h); d) Y = f(X₂,X₃) (X₁ = 2.5 mol L⁻¹, X₄ = 70.0 °C); e) Y = f(X₂,X₄) (X₁ = 2.5 mol L⁻¹, X₃ = 1.5 h); f) Y = f(X₃,X₄) (X₁ = 2.5 mol L⁻¹, X₂ = 20 mL g⁻¹); X₁: acid concentration (mol L⁻¹), X₂: liquid-solid ratio (mL g⁻¹), X₃: extraction time (h), X₄: extraction temperature (°C), Y: yield (mg g⁻¹).

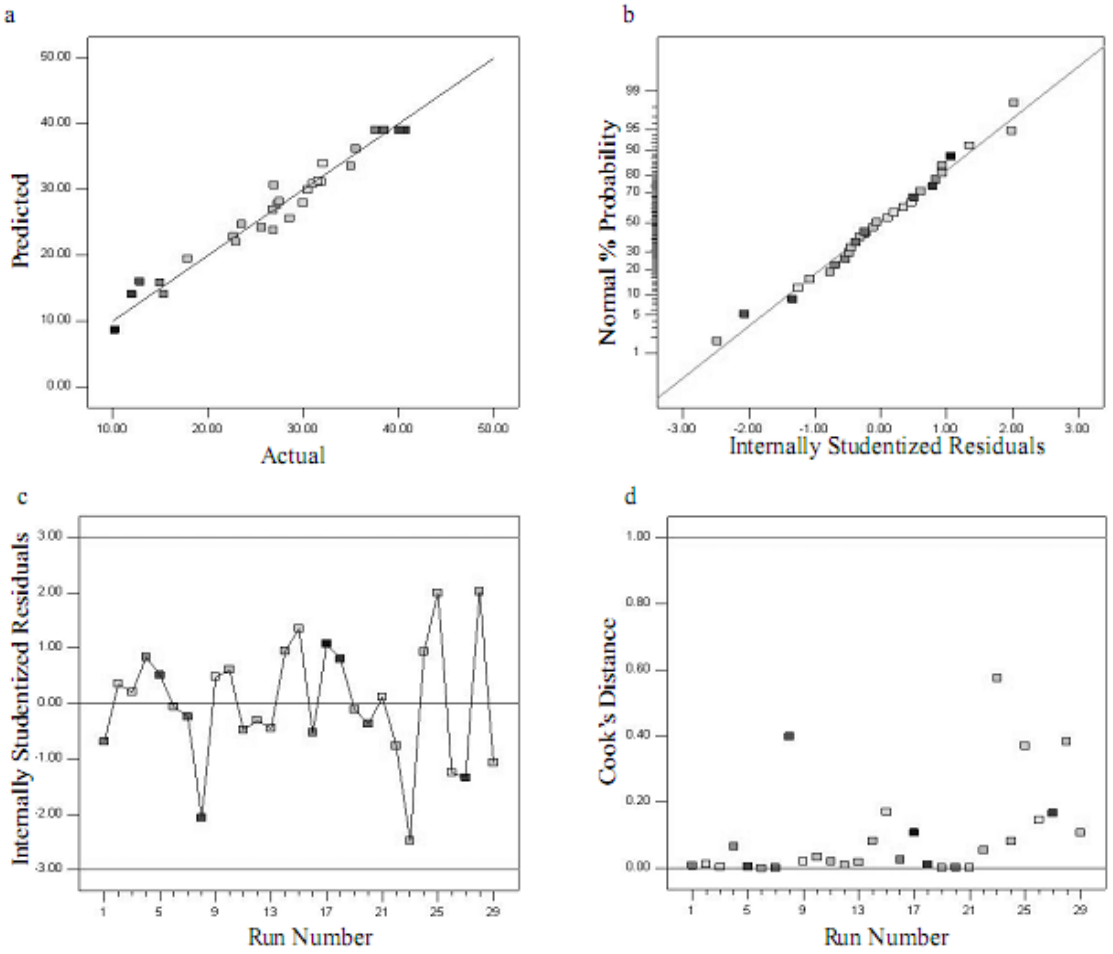

Figure 4. Diagnostic plots for the model adequacy of acid extraction; a) predicted vs. actual, b) normal plot of residuals, c) residuals vs. run and d) Cook's distance.

action term of X_1 and X_3 (acid concentration and extraction time, p = 0.0198 < 0.05) was significant. The other terms were however not significant (p > 0.05). The p-value of model was less than 0.0001 and Adj. R^2 was 0.9281 which would give a better fit to the mathematical model (Eq. (5)).

Verification of the model. The optimum conditions of acid extraction predicted by regression model were acid concentration 2.48 mol L^{-1}, liquid-solid ratio 20.3:1 mL g^{-1}, extraction time 1.56 h and extraction temperature 75.45 °C. Under these optimum extraction conditions, the polysaccharides yield was predicted for 41.41 mg g^{-1}.

The experiment was repeated three times at acid concentration 2.48 mol L^{-1}, liquid-solid ratio 20.3:1 mL g^{-1}, extraction time 1.56 h and extraction temperature 75.5 °C. The average value of practical yield of polysaccharide was 42.13±0.15 mg g^{-1}(n=3), the relative error was 1.74% compared with predicted yield of 41.41 mg g^{-1}. The analysis results confirmed that the second order polynomial equation was adequate for reflecting the expected optimization and the Eq. (5) was satisfactory and accurate.

Effect of acid on yield of polysaccharides

The experimental results showed that the polysaccharides yield of acid extraction was significantly higher than that of hot water extraction. Extraction yield was increased 109.9%. This is because the acid can help eliminate the physical and chemical effect between cell wall of polymer molecules, make more polysaccharides to dissolve from cells into solution in the same extraction time, and thus the yield of polysaccharides is increased. However, high acid concentration would reduce the polysaccharides yield because of the destruction of the structure of polysaccharide caused by the acid catalyzed hydrolysis [8].

Effect of other factors on yield of polysaccharides

The obtained experiment data indicated that liquid-solid ratio, extraction time and extraction temperature of each extraction method were similar, and their trends of influence on polysaccharides yield in

each extraction process were also similar. The yield raised with increasing liquid-solid ratio because the polysaccharides concentration difference between sample and solution was increased to result in the raised of mass transfer driving force. However, if the liquid-solid ratio was too high, it would lead to increased operating costs, and the increased of amount of acid in the extract system would lead to the destruction of the structure of polysaccharide caused by the acid catalyzed hydrolysis. These results are in good agreement with previous observations [8,13-15]. The extraction time had a similar effect on yield as liquid-solid ratio. The yield first increased and then decreased with increasing of the extraction time. This phenomenon could be explained by that the proso millet cell-wall was broken, the liquid was infiltrated into the dried sample, the polysaccharides in sample was dissolved and subsequently diffused out from the sample to exterior solvent, which needed a long time, but the polysaccharides dissolved in solution would be partly degradation because they remained in the solution for a long time [13,16]. The extraction yield was found to increase with increase of extraction temperature, and then decreased after a peak. The reason was that the increased system temperature resulted in the decreased of solvent viscosity to enhance the solvent and solute diffusivity within suspension solution system, which raised the polysaccharides solubility. But the polysaccharide could be degraded under high temperature, so the yield would be decreased [13,15]. There were no obvious differences between Figures 2 and 4, both shown that no obvious patterns were found in the analysis of model and indicated the accuracy of the developed model.

CONCLUSION

The performance of the extraction of polysaccharides from proso millet was studied with a statistical method based on the response surface methodology in order to identify and quantify the variables that may maximize the yield of polysaccharides. The second order polynomial model had higher correlation for experiment data and could be better used for optimizing proso millet polysaccharides extraction technology. The optimum conditions of hot water extraction were liquid-solid ratio 19.8:1 mL g^{-1}, extraction time 1.8 h and extraction temperature 71.8 °C, and the yield was 20.07±0.21 mg g^{-1} (n = 3). The optimum conditions of acid extraction were acid concentration 2.48 mol L^{-1}, liquid-solid ratio 20.3:1 mL g^{-1}, extraction time 1.56 h and extraction temperature 75.5 °C, the polysaccharides yield was 42.13±0.15 mg g^{-1} (n = 3). The acid solution extraction of polysaccharides was better than hot water extraction; the yield of acid solution extraction was higher by 109.9% than of hot water extraction. The optimal extraction conditions of hot water extraction and acid extraction were determined, and under the optimum conditions, the practical yield was agreed closely with the predicted yield value.

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NAUČNI RAD

POREĐENJE EKSTRAKCIJA POLOSAHARIDA IZ PROSA TOPLOM VODOM I KISELIM RASTVOROM

Ispitivana je ekstrakcija polisaharida iz prosa pomoću tople vode i kiseli vodeni rastvor. Za određivanje najbolje moguće kombinacije koncentracije kiseline, odnosa tečno-čvrsto, vremena i temperature ekstrakcije koja obezbeđuje ostvarivanje najvećeg prinosa polisaharida ekstrakcijom sa toplom vodom, odnosno kiselim rastvorom, korišćena je metodologija odgovora površine, Box-Behnken dizajnu sa tri nivo i 3, odnosno 4 faktora. Eksperimentalni podaci su fitovani polinomnom jednačinom drugog reda i analizirani odgovarajućim statističkim metodama, pri čemu su određeni odgovarajući optimalni uslovi obadve ekstrakcione metode. Pod optimalnim uslovima, eksperimentalni prinos se dobro slaže sa vrednostima koje su izračunate modelom. Prinos polisaharida dobijenih kiselom ekstrakcijom iznosi 42,13 mg·g^{-1} i veći je od prinosa ostvarenog ekstrakcijom pomoću tople vode (20,07 mg·g^{-1}). Obe kvadratne jednačine se mogu koristiti za predviđanje rezultata ekstrakcija.

Ključne reči: proso, polisaharidi, ekstrakcija toplom vodom, kisela ekstrakcija, metodologija odgovora površine.