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Optimization of Ultrasonic Extraction of Polysaccharides from Chinese Malted Sorghum Using Response Surface Methodology

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Abstract: Ultrasonic technology was applied for polysaccharides extraction from the Chinese malted sorghum and Response Surface Methodology (RSM) was used to optimize the effects of processing parameters on polysaccharides yields. Three independent variables were ultrasonic power (X_1), extraction time (X_2) and ratio of water to raw material (X_3), respectively. The statistical analysis indicated that three variables and the quadratic of X_1 and X_2 had significant effects on the yields and followed by the significant interaction effects between the variables of X_2 and X_3 ($p<0.05$). A mathematical model with high determination coefficient was gained and could be employed to optimize polysaccharides extraction. The optimal extraction conditions of polysaccharides were determined as follows: Ultrasonic power 600 W, extraction time 4 min, ratio of water to raw material 30 ml/g. Under these conditions, the experimental yield of polysaccharides was 17.08±0.33%, which was agreed closely with the predicted value 17.06%.

Key words: Response surface methodology, malted sorghum, optimization, polysaccharides extraction

INTRODUCTION

As more than 500 million people in the developing countries depend on sorghum as the main staple food, relevant scientific information generated for this crop can certainly play a key role in food development (Mutisya *et al.*, 2009). Recently, there has been increased interest in sorghum as a gluten-free cereal to substitute the gluten-rich cereals in the diet of people suffering from celiac disease. Polysaccharides and lignin have been considered as the main functional compositions and great attentions have been paid for their great health effects (Yang *et al.*, 2008a), such as promoting blood metabolism, soothe nerves, relieve insomnia, etc. (Yang *et al.*, 2008b; Yang *et al.*, 2009). Polysaccharides from plant, epiphyte and animals extracts are an interesting source of additives for several industries, in particular food and drug industry (Forabosco *et al.*, 2006). They play important roles in the growth and development of living organisms and have been widely studied in recent years due to their unique biological, chemical and physical properties (Schepetkin and Quinn, 2006). Whereas, there have been only a few reports on Chinese malted sorghum polysaccharides and few on its functional effects. One of the reasons is the lack of high efficient extraction technology of polysaccharides from Chinese malted sorghum pulp. Hot-water technology is the main extraction method of Chinese malted sorghum polysaccharides in recent research, which is a classical extraction of polysaccharides. It usually requires long extraction time, high temperature and extraction efficiency is low (Li *et al.*, 2007). Therefore, it's essential and desirable to find an economical and high efficient extraction method of polysaccharides from Chinese malted sorghum.

Ultrasonic treatment has been employed for preparing polysaccharides from different plant materials in recent years and showed the great extraction efficiency (Hromadkova *et al.*, 1999; Hromadkova and Ebringerova, 2003; Hofmann *et al.*, 2006; Hemwimon *et al.*, 2007; Wang *et al.*, 2009). This great extraction efficiency by ultrasonic treatment is mainly attributed to its mechanical effects, which greatly facilitate mass transfer between immiscible phases through a super agitation (Vinatoru *et al.*, 1997) and the most important mechanical effects of ultrasonic treatments are microjetting and microstreaming (Tsochatzidis *et al.*, 2001; Velickovic *et al.*, 2006). Response Surface Methodology (RSM) is an affective statistical technique for optimizing complex processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Giovanni, 1983). It is wide used in optimizing the extraction process variables, such as polysaccharides, anthocyanins, vitamin E, phenolic compounds and protein from varied materials (Cacace and Mazza, 2003; Ge *et al.*, 2002; Chandrika and Fereidoon, 2005; Lee *et al.*, 2005; Li and Fu, 2005; Liyana-Pathirana and Shahidi, 2005a; Qiao *et al.*, 2009).

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Box-Behnken Design (BBD), one of RSM, only have three levels and need fewer experiments. It's more efficient and easier to arrange and interpret experiments in comparison with others and widely used by many researches (Box and Behnken, 1960; Ferreira *et al.*, 2007).

In this study, the main objective was to optimize ultrasonic technology conditions for the extraction of polysaccharides from Chinese malted sorghum. RSM was designed to systemic analyze the effects of extraction parameters on the yields of polysaccharides from Chinese malted sorghum and their interactions.

MATERIALS AND METHODS

Experimental materials and chemicals: Sorghum [*Sorghum bicolor* (L.) Moench] was grown in Shandong, a coastal province East of China and known to have average January temperature of 0°C and July 28°C. The average annual rainfall is about 500 mm, most of which falls in the summer. Red sorghum was obtained from 2007 and 2008 harvest. The length/breadth ratio of sorghum kernel was 1.12/1.23 and the density (g/L) was 691.40. The average weight of 1000 kernels was 26.80 g.

All the chemicals used were of analytical grade and purchased from Sinopharm Chemicals Reagent Company (SCRC), Shanghai, China.

Soaking, malting and preparation of sorghum flour: After removing chaff and unviable grain, sorghum grains (1000 g) were thoroughly cleaned by washing with tap water and then soaked in wooden ash extract. The grains were soaked for 24 h at 30°C with the soaking water being changed at 6 h interval. After soaking, the grains were spread on jute bags and covered with the same material in a secluded and dark area. Malting was allowed to proceed for different time intervals (3, 5 and 6 days) and the temperature of malting kernels was 25°C. The growth was terminated by kilning in a forced air oven at 40°C for 24 h. The withered rootless were gently brushed off and dried grain were milled using a bench-top attrition mill (Dade, DFT-600, 25000 rpm, Zhejiang Linda Mechanic Co., Ltd., China). The resultant flour was sieved into a particle size of 70-mesh. The flour was then packaged in a low density polyethylene bag and was stored using plastic containers with lids in a refrigerator at 4°C for later analysis.

Extraction of polysaccharides from Chinese malted sorghum with ultrasonic treatment: The process of polysaccharides extraction from Chinese malted sorghum by ultrasonic treatment was performed in an ultrasonic cell disintegrator (JBT/C-YCL400T, Xinzhi Bio-technology and Science Inc., Lingbo, Henan Province, China). Two grams of Chinese malted sorghum powders were extracted with distilled water in a 100-ml beaker, then the beaker was held in the ultrasonic cell disintegrator and exposed to extract for different time at varied ultrasonic power. Ice bathing was used to ensure the temperature of solution was below 50°C in the whole extraction processing.

Isolation and determination yield of Chinese malted sorghum polysaccharides: After the extraction with ultrasonic treatment, the extracted slurry was centrifuged at 10000 rpm/min for 20 min to collect the supernatant, and the insoluble residue was treated again for 2-3 times as mentioned above. The supernatant was incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (SENCO Technology and Science Inc., Shanghai, China) at 55°C under vacuum. The resulting solution was mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) and kept overnight at 4°C. Then the solution was centrifuged at 10000 rpm/min for 20 min, washed six times with dehydrated ethanol and the precipitate was collected as crude extract. The extract was air-dried at 50°C until its weight was constant and then was weighted with a balance (AY 120, SHIMADZU, Japan). The percentage polysaccharides yield (%) was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of dried crude extractraction (g)}}{\text{Weight of malted sorghum powder (g)}} \times 100 \quad (1)$$

Experimental design: A three level, three variable Box-Behnken Factorial Design (BBD) (Design Expert software, Version 6.0.5, Stat-Ease Inc., Minneapolis, MN) was applied to determine the best combination of extraction variables for the yields of Chinese malted sorghum polysaccharides. Three extraction variables considered for this research were X_1 (ultrasonic power), X_2 (extraction time) and X_3 (ratio of water to raw material) (Li *et al.*, 2007) and the proper range of three variables were determined on the basis of single-factor experiment for the polysaccharides production (Table 1). Table 1 listed the whole design consisted of 17 experimental points, five replicates (treatment 13-17) at the centre of the design were used to allow for estimation of a pure error sum of squares. The triplicates were performed at all design points in randomized order. Experimental data were fitted to a quadratic polynomial model and regression coefficients obtained. The non-linear computer generated quadratic model used in the response surface was as follows:

$$Y = \beta_0 + \sum_{i=0}^{4}\beta_i X_i + \sum_{i=0}^{4}\beta_{ii} X_i^2 + \sum_{i=0}^{4}\sum_{j=0}^{4}\beta_{ij} X_i X_j \quad (2)$$

Where Y is the measured response associated with each factor lever combination; β_0 is an intercept; β_i is

Table 1: Box-Behnken experimental design with the independent variables

	Valuables levels			Polysaccharides yield (Y) (%)	
Run	X_1	X_2	X_3	Experimental	Predicted
1	-1(500)	-1(3.5)	0(30)	11.01±0.32	10.88
2	1(700)	-1(3.5)	0(30)	16.15±0.26	16.23
3	-1(500)	1(4.5)	0(30)	11.89±0.61	11.83
4	1(700)	1(4.5)	0(30)	16.70±0.11	16.83
5	-1(500)	0(4.0)	-1(25)	11.78±0.08	11.92
6	1(700)	0(4.0)	-1(25)	17.26±0.32	17.21
7	-1(500)	0(4.0)	1(35)	11.66±0.24	11.72
8	1(700)	0(4.0)	1(35)	16.90±0.43	16.76
9	0(600)	-1(3.5)	-1(25)	16.12±0.56	16.11
10	0(600)	1(4.5)	-1(25)	17.48±0.16	17.42
11	0(600)	-1(3.5)	1(35)	16.26±0.22	16.33
12	0(600)	1(4.5)	1(35)	16.54±0.09	16.56
13	0(600)	0(4.0)	0(30)	17.36±0.24	17.06
14	0(600)	0(4.0)	0(30)	17.03±0.93	17.06
15	0(600)	0(4.0)	0(30)	16.97±1.67	17.06
16	0(600)	0(4.0)	0(30)	17.08±0.33	17.06
17	0(600)	0(4.0)	0(30)	16.88±0.41	17.06

regression coefficients computed from the observed experimental values of Y and X_i is the coded levels of independent variables. The terms $X_i X_j$ and X_i^2 represent the interaction and quadratic terms, respectively.

Statistical analyses: Data were expressed as means Standard Errors (SE) of three replicated determinations. The responses obtained from each set of experimental design (Table 1) were subjected to multiple non-linear regressions using the Design Expert software (Version 6.0.5, Stat-Ease Inc., Minneapolis, MN). The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2 and the significance of the regression coefficient were checked by F-test and p-value.

RESULTS AND DISCUSSION

Fitting the model: A regression analysis (Table 2) was carried out to fit mathematical models to the experimental data aiming at an optimal region for the responses studied. Predicted response Y for the yield of Chinese malted sorghum polysaccharides could be expressed by the following second order polynomial equation in terms of coded values:

$$Y = 17.06 + 2.59X_1 + 0.39X_2 - 0.16X_3 - 2.66X_1^2 - 0.46X_2^2 + 2.5.10^{-3}X_3^2 - 0.088X_1X_2 - 0.060X_1X_3 - 0.27X_2X_3 \quad (3)$$

Where Y is the yield of Chinese malted sorghum polysaccharides (g) and X_1, X_2 and X_3 are the coded variables for ultrasonic power, extraction time and the ratio of water to the raw material, respectively.

In general, exploration and optimization of a fitted response surface may produce poor or misleading results, unless the model exhibits a good fit, which makes checking of the model adequacy essential (Liyana-Pathirana and Shahidi, 2005b). The F-ratio in this table is the ratio of the mean square error to the pure error obtained from the replicates at the design centre. The significance of the F-value depends on the number of Degrees of Freedom (DF) in the model, and is shown in the p-value column (95% confidence level). Thus, the effects lower than 0.05 in this column are significant (Cai *et al*., 2008; Qiao *et al*., 2009).

Table 2 listed the Analysis of Variance (ANOVA) for the fitted quadratic polynomial model of extraction of Chinese malted sorghum polysaccharides.

F-test suggested that model had a very high model F-value (F = 302.64) and a very low p-value ($p<0.0001$); indicating this model was highly significant. The lack of fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. As showed in Table 2, F-value and p-value of the lack of fit were 0.91 and 0.5103, respectively, which implied it was not significant relative to the pure error and indicated that the model equation was adequate for predicting the Chinese malted sorghum polysaccharides under any combination of values of the variables. R^2 adj (adjusted determination coefficient) is the correlation measure for testing the goodness-of-fit of the regression equation. Higher it is the better degree of correlation between the observed and predicted values (Ravikumar *et al*., 2006). The value of R^2 adj for Eq. (3) was 0.994, which was reasonably close to 1 and implied that only less 1.0% of the total variations were not explained by model.

Meanwhile, it also confirmed that the model was highly significant and indicated a high degree of correlation between the observed and predicted data. Coefficient of Variation (CV) indicates the degree of precision with which the experiments are compared. A relatively low value of CV (1.14) in Table 2, which showed a better precision and reliability of the experiments carried out.

Table 2: Analysis of variance for the fitted quadratic polynomial model of extraction of polysaccharides

Source	SS	DF	MS	F-value	Prob>F
Model	86.6861	9	9.6300	302.6419	<0.0001
Residual	0.2227	7	31.8257×10^{-3}		
Lack of fit	90.524×10^{-3}	3	30.17467×10^{-3}	0.91	0.5103
Pure error	132.256×10^{-3}	4	33.0640×10^{-3}		
Cor Total	86.9089	16			
	$R^2 = 0.997$	R^2adj = 994	CV = 1.14		

SS: Sum of Squares; DF: Degree of Freedom; MS: Mean Square

Table 3: Estimated regression model of relationship between response variables (yield of Chinese malted sorghum polysaccharides) and independent variables (X_1, X_2, X_3)

Variables	DF	SS	MS	F-Value	p-Value
X_1	1	53.4578	53.4578	1679.7046	<0.0001
X_2	1	1.1889	1.1889	37.3560	0.0005
X_3	1	0.2060	0.2060	6.4753	0.0384
X_1X_1	1	29.8480	29.8480	937.8588	<0.0001
X_2X_2	1	0.8890	0.8890	27.9337	0.0011
X_3X_3	1	2.6315×10^{-5}	2.6315×10^{-5}	8.2687×10^{-4}	0.9779
X_1X_2	1	0.0309	0.0309	0.9733	0.3567
X_1X_3	1	0.0144	0.0144	0.4524	0.5227
X_2X_3	1	0.2981	0.2981	9.3671	0.0183

SS: Sum of Squares; DF: Degree of Freedom; MS: Mean Square

The significance of each coefficient was determined using p-value in Table 3. The p-value is used as a tool to check the significance of each coefficient and the interaction strength between each independent variable. The corresponding variables would be more significant at greater F-value and smaller p-value (Atkinson and Donev, 1992). The data in the Table 3 indicated that all the independent variables (X_1, X_2, X_3) and two quadratic terms (X_{12} and X_{22}) significantly affected the yield of Chinese malted sorghum polysaccharides and there was significant interaction between extraction time (X_2) and ratio of water to raw material (X_3). Meanwhile, the ultrasonic power (X_2) was the major factor affecting the yield of polysaccharides.

Analysis of response surface: The 3D response surface and 2D contour plots are the graphical representations of regression equation. They provide a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. Circular contour plot indicates that the interactions between the corresponding variables are negligible, while elliptical contour plot indicates that the interactions between the corresponding variables are significant.

The relationship between independent and dependent variables was illustrated in tri-dimensional representation of the response surfaces and two-dimensional contour plots generated by the model for yield of polysaccharides (Fig. 1-3), two variables were depicted in one tri-dimensional surface plots while the other variable kept at level zero. It is clear that the yield of polysaccharides was sensitive to minor alterations of the test variables (ultrasonic power, extraction time and ratio of water to raw material).

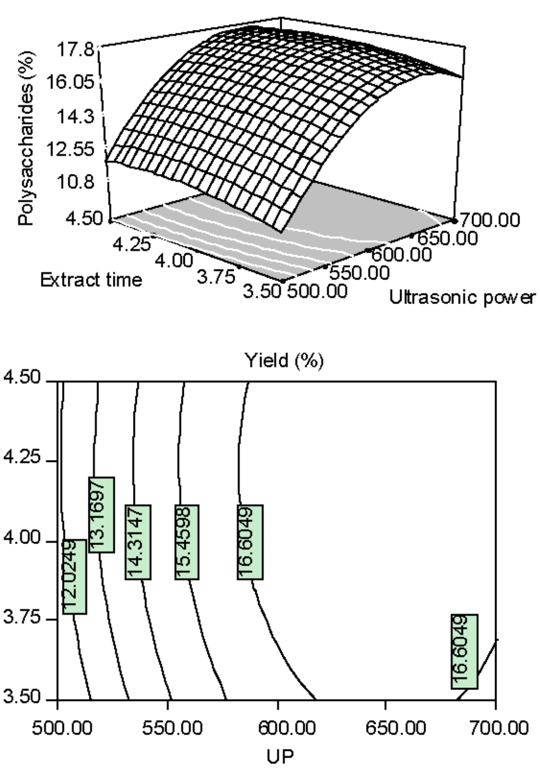

Fig. 1: Response surface plot and contour plot of Ultrasonic Power (UP) and Extraction Time (ET) and their mutual interactions on the yield of Chinese malted sorghum polysaccharides

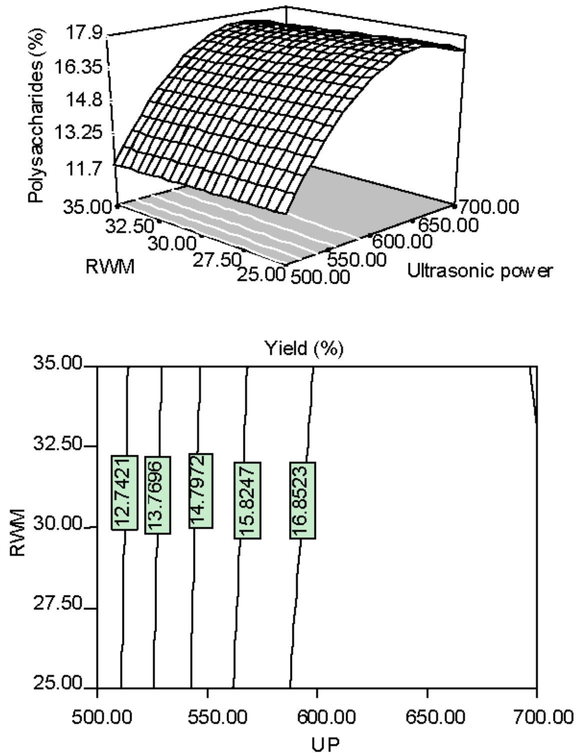

Fig. 2: Response surface plot and contour plot of Ultrasonic Power (UP) and the Ratio of Water to Material (RWM) and their mutual interactions on the yield of Chinese malted sorghum polysaccharides

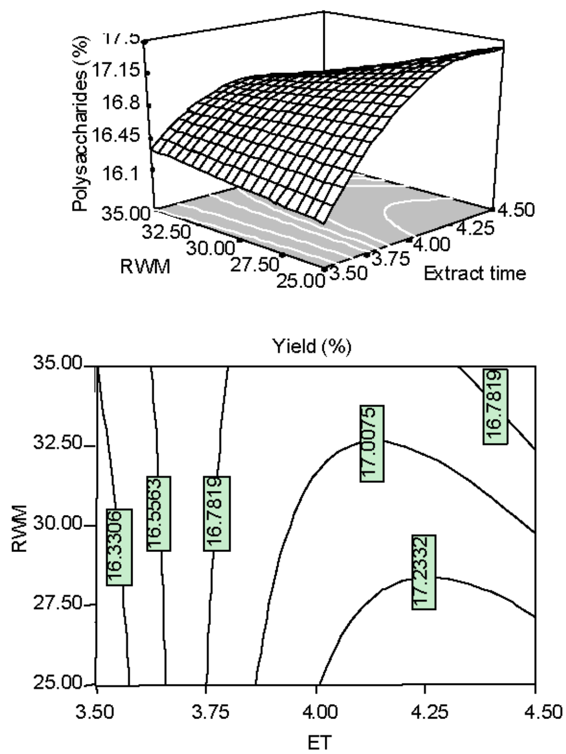

Fig. 3: Response surface plot and contour plot of Extraction Time (ET) and the Ratio of Water to Material (RWM) and their mutual interactions on the yield of Chinese malted sorghum polysaccharides

The interaction relationships of ultrasonic power (X_1) with the extraction time (X_2) and radio of water to material (X_3) on the yield of polysaccharides were shown in Fig. 1 and 2, respectively and indicated that these three variables had all significant effect on the yield of Chinese malted sorghum polysaccharides. As shown in Fig. 1 and 2, the ultrasonic power (X_1) and extraction time (X_2) had positive impact on the polysaccharides production, while the yield changed slightly when the ratio of water to material (X_3) was in the range of 25-35 ml/g. Yield of polysaccharides rapid enhanced with the increasing of ultrasonic power (X_1) and reached to the peak value at 600 W. With the farther increasing of ultrasonic power (X_1), the yield went to slight decrease. Longer extraction time (X_2) had positive effects on the yield extraction and had a critical value at 4 min when at a constant ultrasonic power (600 W). This suggested more yield was resulted at higher ultrasonic power, longer extraction time and lower ratio of water to material.

It was considered higher extraction efficiency of polysaccharides at higher ultrasonic power due to the increase in the number of cavitations bubbles formed and enhance mass transfer rates. However, less yield was resulted at farther increasing ultrasonic power. The same result was gained by Li *et al.* (2007) and was considered for that a part of polysaccharides could be more depolymerize into some free sugars.

It was shown that the interactions between the ultrasonic power and other two extraction variables did not impact the yield of polysaccharides significantly (Table 3, Fig. 1 and 2), in spit of the ultrasonic power was the major factor affecting the yield of polysaccharides. This observation was in agreement with previous investigation. Li *et al.* (2007) researched the optimization of the ultrasonically assisted extraction of polysaccharides from Zizyphus jujuba cv. Jinsixiaozao by RSM and analyzed the effects of interactions of extraction variables on the yield of polysaccharides. They also indicated that the interactions between ultrasonic power and extraction time and ultrasonic power and ratio of water to material caused no significant effect on the extraction yield, while ultrasonic power had significant effect. Fig. 3 showed the response surface plot at various extraction times (X_2) and ratio of water to material (X_3). The response curves demonstrated that higher yield at longer extraction time. The response curves were comparatively smooth at lower extraction time, indicating the less effect on the increasing of the yields extraction when ratio of water to material changed in the range from 25-35 ml/g. However, the yield decreased with the farther enhancing of ratio of water to material at longer extraction time. This result indicated that extraction time (X_2) had a different extent of influence on extraction yield in different ratio of water to materials (X_3), and significant interactions were existed between extraction time (X_2) and ratio of water to raw material (X_3). Higher yields of polysaccharides were resulted at longer extraction time and lower ratio of water to material in the experimental range.

As shown in Fig. 3 and Table 3, the interactions of extraction time and ratio of water to materials had significant effect on the extraction yields, which was the same with other research results (Rodrigues *et al*., 2008; Wang *et al*., 2009). This conclusion was inconsistent with the observation obtained by Li *et al.* (2007), who reported that this interaction caused no significant effect on the extraction yields at a constant ultrasonic power. This contradiction was possibly due to the large difference in parameters of sonic power. In this study, the sonic power (>500 W) were far greater than that (60 W) in Li *et al.* (2007).

Confirmative tests: The suitability of the model equation for predicting the optimum response value was tested using the recommended optimum conditions. Optimum values of independent variables (Ultrasonic power of 600 W, extraction time of 4 min and ratio of water to material 30 ml/g.) were incorporated into the regression equation. A mean value of 17.064±0.181% (N = 5) was gained and was in agreement with the predicted value significantly ($p>0.05$) obtained from real experiments which demonstrated the validation of the RSM model. The results of analysis confirmed that the response model was adequate for reflecting the expected optimization and the model of Eq. (3) was satisfactory and accurate.

Conclusion: Ultrasonic technology was performed for the polysaccharides extraction from Chinese malted sorghum in order to increase the yield extraction. The experimental value of polysaccharides yield varied from 11.01-17.48% of malted sorghum. Based on the single-factor experiments, Response Surface Methodology (RSM) was used to estimate and optimize the experimental variables-ultrasonic power, extraction time and ratio of water to raw material. All the independent variables, quadratic of ultrasonic power and extraction time had high significant effects on the response values, followed by the significant interaction effects between the extraction time and ratio of water to material. A high correlation of the quadratic polynomial mathematical model was gained and could be great employed to optimize polysaccharides extraction from Chinese malted sorghum by ultrasonic technology. The optimal extraction conditions for the polysaccharides were determined as follows: Ultrasonic power 600 W, extraction time 4 min, ratio of water to material 30 ml/g. Under these conditions, the experimental yield of polysaccharides was 17.08±0.33%, which was agreed closely with the predicted yield value.

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REFERENCES

Atkinson, G.L. and A.N. Donev, 1992. Optimum Experimental Design. Oxford: Clarendon Press.

Box, G.E.P. and D.W. Behnken, 1960. Some new three level designs for the study of quantitative variables. Technometrics, 2: 455-475.

Cacace, J.E. and G. Mazza, 2003. Optimization of extraction of anthocyanins from black currants with aqueous ethanol. J. Food Sci., 68: 240-248.

Cai, W.R., X.H. Gu and J. Tang, 2008. Extraction, purification and characterization of the polysaccharides from Opuntia milpa alta. Carbohyd. Polym., 71: 403-410.

Chandrika, L.P. and S. Fereidoon, 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem., 93: 47-56.

Ferreira, S.L.C., R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David and G.C. Brand, 2007. Box–Behnken design: An alternative for the optimization of analytical methods. Analytica Chimica Acta, 597: 179-186.

Forabosco, A., G. Bruno, L. Sparapano, G. Liut, D. Marino and F. Delben, 2006. Pullulans produced by strains of Cryphonectria parasitica-I. Production and characterisation of the exopolysaccharides. Carbohyd. Polym., 63: 535-544.

Ge, Y., Y. Ni, H. Yan, Y. Chen and T. Cai, 2002. Optimization of the supercritical fluid extraction of natural vitamin E from wheat germ using response surface methodology. J. Food Sci., 67: 239-243.

Giovanni, M., 1983. Response surface methodology and product optimization. Food Technol., 45: 3741.

Hemwimon, S., P. Pavasant and A. Shotipruk, 2007. Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia. Sep. Purif. Technol., 5444: 5540.

Hofmann, R., T. Kappler and C. Posten, 2006. Pilot-scale press electrofiltration of biopolymers. Sep. Purif. Technol., 51: 303-309.

Hromadkova, Z. and A. Ebringerova, 2003. Ultrasonic extraction of plant materials-Investigation of hemicellulose release from buckwheat hulls. Ultrason. Sonochem., 10: 127-133.

Hromadkova, Z., A. Ebringerova and P. Valachovic, 1999. Comparison of classical and ultrasound-assisted extraction of polysaccharides from *Salvia officinalis* L. Ultrason. Sonochem., 5163: 168.

Lee, G.D., J.O. Kim and J.H. Kwon, 2005. Optimum conditions for the extraction of effective substances from the stem of Opuntia fiscus-indica. Food Sci. Biotechnol., 14: 190-195.

Li, J.W., S.D. Ding and X.L. Ding, 2007. Optimization of the ultrasonically assisted extraction of polysaccharides from *Zizyphus jujuba* cv. Jinsixiaozao. J. Food Eng., 80: 176-183.

Li, Q.H. and C.L. Fu, 2005. Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. Food Chem., 92: 701-706.

Liyana-Pathirana, C.M. and F. Shahidi, 2005a. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivium* L.) as affected by gastric pH conditions. J. Agric. Food Chem., 53: 2433-2440.

Liyana-Pathirana, C.M. and F. Shahidi, 2005b. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem., 93: 47-56.

Mutisya, J., C. Sun, S. Rosenquist, Y. Baguma and C. Jansson, 2009. Diurnal oscillation of SBE expression in sorghum endosperm. J. Plant Physiol., 166: 428-434.

Qiao, D.L., C.L. Kea, B. Hua, J.G. Luo, H. Ye and Y. Sun, 2009. Antioxidant activities of polysaccharides from Hyriopsis cumingii. Carbohyd. Polym., 78: 199-204.

Ravikumar, K., S. Ramalingam, S. Krishnan and K. Balu, 2006. Application of response surface methodology to optimize the process variables for reactive red and acid brown dye removal using a novel absorbent. Dyes Pigments, 70: 18-26.

Rodrigues, S., G.A.S. Pinto and F.A.N. Fernandes, 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder by response surface methodology. Ultrason. Sonochem., 15: 95-100.

Schepetkin, I.A. and M.T. Quinn, 2006. Botanical polysaccharide: Macrophage immunomodulation and therapeutic potential. Int. Immunopharmacol., 6: 317-333.

Tsochatzidis, N.A., P. Guiraud, A.M. Wilhelm and H. Delmas, 2001. Determination of velocity, size and concentration of ultrasonic cavitation bubbles by the phase-Doppler technique. Chem. Eng. Sci., 56: 1831-1840.

Velickovic, D.T., D.M. Milenovic, M.S. Ristic and V.B. Veljkovic, 2006. Kinetics of ultrasonic extraction of extractive substances from garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage. Ultrason. Sonochem., 13: 150-156.

Vinatoru, M., M. Toma, O. Radu, P.I. Filip, D. Lazurca and T.J. Mason, 1997. The use of ultrasound for the extraction of bioactive principles from plant materials. Ultrason. Sonochem., 4: 135-139.

Wang, Y.J., Z. Cheng, J.W. Mao, M.G. Fan and X.Q. Wu, 2009. Optimization of ultrasonic-assisted extraction process of Poria cocos polysaccharides by response surface methodology. Carbohyd. Polym., 77: 713-717.

Yang, B., M.M. Zhao, J. Shi, N. Yang and Y.M. Jiang, 2008a. Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. Food Chem., 106: 685-690.

Yang, C.X., N. He, X.P. Ling, M.L. Ye, C.X. Zhang and W.Y. Shao, 2008b. The isolation and characterization of polysaccharides from longan pulp. Sep. Purif. Technol., 63: 226-230.

Yang, B., Y.M. Jiang, R. Wang, M.M. Zhao and J. Sun, 2009. Ultra-high pressure treatment effects on polysaccharides and lignins of longan fruit pericarp. Food Chem., 112: 428-431.