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Full Length Research Paper

Optimization of Microwave Extraction of Polysaccharides from Stigma Maydis by Central Composition Design

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Polysaccharides extraction from *Stigma Maydis* was carried out using microwave- directed. The Plackett-Burnam design was applied to screening the key factors which would affect the yield of Stigma Maydis polysaccharides extracted. Key factors were microwave-directed extracting temperature, extracting power, and liquid-solid ratio. The path of steepest climbing was used to approach the optimal region of the extraction condition, and the experiment parameters were further optimized using central composite design and response surface analysis, the optimized condition was obtained. The statistical analysis of the experiment indicated that the extracting temperature, the power had significant effect on the polysaccharides yield. The central composite design showed the polynomial regression models were in good agreement with the experimental results for the polysaccharides yield. The optimized condition for polysaccharides yield within the experimental range of the variables studied was extracting temperature 85 ℃, power 400 W, liquid-solid ratio 80mL/g, time 15 min. At this condition, the predicted polysaccharides yield was 8.93±0.43%, the real value was 9.36±0.22%, and the relative deviation between the predicted valued and real value was less than 0.5%.

Keywords: Microwave, *Stigma Maydis* Polysaccharides, Plackett-Burnam Design, Central Composition Design, Response Surface Method.

INTRODUCTION

Stigma Maydis is a traditional Chinese herbal medicines, and exhibited weight loss(Du and Xu, 2007) , urinary ailments(Maksimovic, Dobric, Kovacevic and Milovanovic, 2004), anti-fatigue activity(Hu, Zhang, Li, Ding and Li, 2010), anti-tumor activities(Habtemariam, 1998), hypoglycemic effect(Guo, Liu, Han & Liu, 2009), antioxidant activity (Ebrahimzadeh, Pourmorad & Hafezi, 2008),and anti-inflammation (El-Ghorab, El-Massry and Shibamoto,2007b).Chemical analysis revealed that Stigma Maydis consists of a variety of nutritive materials, including polysaccharides(Wei, Lin and Zhu, 2007), flavonoids(Zhou, Zhang, Zhai and Jia, 2009) , isorhamnetin(Cao, Wei and Ito, 2009) ,alkaloids, and tannins(Lin, Chu, Tian and Ye, 2007; Maksimovic, Dong and Cai, 2004) etc. Research has shown that flavonoid have exhibited the anti-fatigue activity (Hu, Zhang, Li, Ding and Li, 2010), phenolic acids and resembling monosides from Stigma Maydis have exhibited the antioxidative activity(Maksimovic and Kovacevic, 2003).The polyphenol classes yields had a positive linear correlation with antioxidant activity (Maksimovic, Malencic and Kovacevic, 2005) and 36 compounds which exhibited clear antioxidant activities have been identified by GC-MS(El-Ghorab, El-Massry and Shibamoto, 2007a). However, polysaccharides constituents still remained uncertain because of its complexity. Therefore, it is important to study about the extraction of polysaccharides from Stigma Maydis, to obtain much higher yield of the polysaccharides.

Polysaccharide, a kind of carbohydrate polymer consisting of many units of monosaccharides, is widely distributed in edible plants and Chinese herbs (Lin, Wang, Chang, Inbaraj and Chen, 2009), and has received

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considerable attention in the past decade because of its vital biological activity, including antibiotic (Maksimovic and Kovacevic, 2003), antitumor(Xu, Yao, Sun & Wu, 2009), and anti-glycated activity (Yang, Zhao & Jiang, 2009).Therefore, polysaccharides from different sources have been emerged the important bioactive natural products(Chen, Meng, Liu, Chen & Zhang, 2010; Cho, Yang, Kim & You, 2010; Zhao, Dong, Chen & Hu, 2010). The contents of polysaccharides in Stigma Maydis are much higher than the flavonoids and other active component researched. The conventional extraction of water-soluble polysaccharides are often carried out by hot water at 95~100℃. Enzyme treatment, transonic, microwave-assistant and a combination of the two processes for the extraction of Stigma Maydis polysaccharides have been studied(Wei, Lin and Zhu, 2007). These procedures are time-consuming and heat energy- consuming. Ultrasonic technology was employed to extract polysaccharide from jujube(Li, Fan, Ding and Ding, 2007)and longan fruit pericarp(Yang et al., 2008) , and the optimal extracted condition of extract polysaccharide was obtained by response surface methodology (RSM).Therefore, a heat treatment by microwave-directed to carry out this extraction procedure instead of a conventional extraction methods is necessary.

In the current paper, optimization of microwave-directed extraction of polysaccharides from *Stigma Madis* was investigated. The first objective of the present study was to determine basic compositions of *Stigma Maydis* .The second objective was to optimize conditions of the extracting temperature, power, liquid-solid ratio and build an extracted model by microwave- directed. Consequently, the compare with traditional hot water extraction and microwave- directed extraction will be discussed. The aim of the paper is to obtain the higher yield of polysaccharides and build a model of extraction technology by microwave directed.

MATERIALS AND METHODS

Materials and Instrumentations

Stigma Maydis used for this investigation were 958 species for Zheng Dan. Mature *Stigma Maydis* of zea mays L, poaceae female flowers gathered from corn fields in Chang chun (China) in January 2008. Collected *Stigma Maydis* were dried in a shaded and well-ventilated place, pulverized in a knife mill and sieved into particle size with mean diameter, then stored in a dry environment before further processing.

Determination of polysaccharides yield

The polysaccharides of *Stigma Maydis* were determined using a phenol–sulphuric acid method as described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). The polysaccharide was calculated based on the standard curve of glucose, which was prepared by using six concentrations of 10, 20, 30, 40, 50 and 60 μg/mL.

Determination of basic composition of *Stigma Maydis*

The basic components in *Stigma Maydis*, including protein, reducing sugar, fat, moisture and ash ,were determined using AOAC official method (1997) (Wang, Chang & Chen, 2009), and the mineral element was analyzed by ICP-MS(Agilent 7500a).

The design of Plackett-Burman experiment

For the highest yield of polysaccharides obtained , the most important variables were selected in the experiment. A total of 11 (k) variables (Table 1), including 6 variables (the extracting temperature, liquid-solid ratio, power, time, particle size and material origin , which maybe affect the yield of polysaccharides) were studied in 12 (N or k + 1)experiments (runs) via the Plackett–Burman design(Chodok, Kanjana-Opas and Kaewsuwan, 2010).Each variable was tested at two levels, high (+) and low (-).The statistical analysis software Minitab 15.1.0 was used to generate the Plackett–Burman experimental design.

All the experiments were performed in triplicate on two separate occasions and the responses are reported as the mean of these responses. The technique of Plackett-Burman is based on the first-order polynomial model:

$$Y = \beta_0 + \sum \beta_i X_i \quad \text{(i=1, 2...... k)}$$

Where Y is the estimated response (polysaccharides yield), β_0 is model intercept, β_i is the regression coefficient, X_i is the level of the independent variable, k is number of variables. The effects of each variable were determined by following standard equation:

$$E_{(Xi)} = \frac{2[\sum R_i^+ - \sum R_i^-]}{N}$$

Where $E_{(X_i)}$ is the effect of the tested variable. R_i^+ and R_i^- are responses (polysaccharides yield) when variables were at high and low levels, respectively. N is total number of experiments or runs (N = 12). Experimental error was estimated by calculating the variance among the dummy variables as follows:

$$V_{eff} = \frac{\sum (E_d)^2}{n}$$

Where V_{eff} is the variance of the effect of high/low levels of variable, E_d is the effect of high/low levels of dummy variable and n is the number of dummy variables. The standard error (SE) of the high/low levels of variable is the square root of the variance of an effect, and the significance level (p value) of each effect of high/low levels of variable was determined using the Student's t test:

$$t_{(X_i)} = \frac{E_{(X_i)}}{SE}$$

where $E_{(X_i)}$ is the effect of the variable X_i . The variables at or above the 85% confidence level (p<0.15) were considered to have significant effects on responses (polysaccharides yield).

The steepest climbing experiment

The fitting equation obtained by CCD can reflect the real situation, if the factors scope selected is far away from the studied, the model

Table 1. Factors and levels of Plackett-Burman experimental design

	A	B	C	D	E	F
Factor name	Temperature（℃）	Liquid-Solid ratio	Power(W)	Time(min)	Particle size	Origin
level（-1）	30	20	300	5	20	siping
level（+1）	100	100	700	30	100	Nong'an

Table 2. Factors and levels of Central Composite Design experimental

Coding and the level	Independent variable factor Temperature（℃）	Power（W）	Liquid-Solid ratio
	X_1	X_2	X_3
-1	65	400	65
0	75	500	75
+1	85	600	85

equation was meaningless. Therefore, the significant factors selected and the trend changed were obtained by the Plackett-Burman experiments design(Hochkirchen, 2009). The direction changed of experimental value was the gradient direction for climbing, that value of step size determined was based on various factors value determined, and near the largest yield of *Stigma Maydis* polysaccharides. According to the results of PB design experiment, the extracting temperature (℃), liquid-solid ratio (mL/g), and power (W) were significant changed in the optimal region, so that the indicator values were closest to the predictive value. According to the effect of main factors on the trend changed of polysaccharides yield, the optimal yield value of polysaccharides extracted was selected by the steepest climbing experimental design.

The Central Composite Design

Base on the effect of PB design for the polysaccharides yield, proper ranges of the extracting temperature, power, and liquid-solid ratio were preliminarily determined. The central composite design was used to build the extraction model. Factors and levels of CCD experimental were showed in Table 2.The main factors and their interactions can be evaluated. By establishing the relationship between fitting factors of the experimental and regression equation , the accurate and effective conclusion of the experiment can be obtained(Liu, Miao, Wen & Sun, 2009).CCD in the experimental design consists of twelve factorial points and three replicates of the central point(Sun, Liu & Kennedy, 2010). Extraction temperature (X_1), extraction power (X_2) and liquid-solid ratio (X_3) were choosed for independent variables. Five replicates central experiment were designed to estimate sum of pure error squares(Fan, Chen & Wang, 2010). The response value in each trial was average of duplicates.

The variables were coded according to the equation:

$$x_i = (X_i - X_0) / \Delta X$$

Where x_i the coded value of the variable is X_i , X_0 is the value of X_i at the center point, and is the step change. The behavior of the system was explained by the following quadratic equation:

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j$$

Where Y is the response variable(polysaccharides in real value), A_0 is constant, and A_i , A_{ii} ,and A_{ij} are coefficients estimated by the model, and the X_i , X_j are independent variables($i \neq j$).The coefficients of the second polynomial model and the responses obtained from each set of experimental design were subjected to multiple nonlinear regressions using software Design-Expert7.0.The significances of the regression coefficients were also tested by F-test.

Conventional extraction methods

Traditional extraction methods are reported in the literature, such as hot water extraction method(Yang et al., 2008), enzyme extraction method(Ducasse et al., 2009) and ultrasonic -assistance extraction method(Zhong & Wang, 2010). In the hot water extraction method, samples were prepared by mixing 2 g of *Stigma Maydis* with deionized water at 100℃, 60min. In the ultrasonic -assistance extraction method(Chen, Gu, Huang, Li, Wang and Tang, 2010; Deng, Li & Yuan, 2006; Wei, Lin and Zhu, 2007) , samples were prepared by mixing 2 g of *Stigma Maydis* with 100mL deionized water at 60℃, 20min. And enzyme extraction method, samples were prepared by mixing 2 g of *Stigma Maydis* with 6.0 ml enzyme, at 50℃. After 170 min, the extracts were filtered and prepared to determine the polysaccharide.

Results and Discussion

Basic composition of *Stigma Maydis*

The basic components of *Stigma Maydis* sample contained reducing sugar (1.35%), followed by, moisture

Table 3. Mineral elements contents of *Stigma Maydis*

Mineral Elements	Content (mg/kg)		Linear Equations	Correlation Coefficient
	1-1	1-2		
Li	0.220	0.202	$Y=6.681\times10^{7}X-8.275\times10^{4}$	R=0.9998
Na	1740.297	1694.462	$Y=4.158\times10^{7}X-2.332\times10^{6}$	R=0.9981
Mg	6536.762	6406.545	$Y=2.572\times10^{7}X-1.736\times10^{5}$	R=0.9998
K	45392.868	44540.592	$Y=3.677\times10^{7}X-2.074\times10^{6}$	R=0.999
Ca	5648.470	5432.662	$Y=1.735\times10^{4}X+1.616$	R=0.9992
Mn	149.669	143.581	$Y=4.164\times10^{7}X-4.343\times10^{5}$	R=0.9998
Fe	9267.908	8867.212	$Y=1.508\times10^{5}X-3.146$	R=0.9996
Cu	11.157	10.492	$Y=3.136\times10^{6}X+2.675\times10^{3}$	R=1
Zn	99.858	98.238	$Y=6.246\times10^{5}X-3.098\times10^{3}$	R=0.9999
As	1.201	1.175	$Y=7.483\times10^{5}X-4.822\times10^{2}$	R=0.9979
Se	0.314	0.255	$Y=5.636\times10^{4}X-2.518\times10^{1}$	R=0.999
Sr	46.971	44.698	$Y=4.953\times10^{7}X-9.477\times10^{5}$	R=0.9995
Ag	0.068	0.058	$Y=4.500\times10^{6}X-1.153\times10^{3}$	R=0.9992
Cd	0.110	0.096	$Y=8.551\times10^{5}X+4.705\times10^{-1}$	R=0.9985
Ba	22.420	21.397	$Y=1.340\times10^{6}X-7.514\times10^{2}$	R=1
Hg	0.078	0.078	$Y=1.384\times10^{6}X+3.779\times10^{-1}$	R=0.9978
Pb	3.780	3.463	$Y=1.643\times10^{7}X-1.371\times10^{4}$	R=0.9991

(9.97%), protein (15.75%), ash (10.28%) and fat (0.59%).The mineral element contents of *Stigma Maydis* were showed in the Table.3.

The PB design experimental results

The response value of *Stigma Maydis* polysaccharides yield affected by the six main factors was investigated in Table 1, the effect of extracting temperature, liquid-solid ratio, power were significant. There was an increasing trend in the polysaccharides yield from 30 to 100 ℃ of extracting temperature, 20mL/g to 100mL/g of liquid-solid ratio. Under high extracting temperature and liquid-solid ratio, more solvent will enter into the cell and more compounds can permeate the cell membrane easily. This tendency was in agreement with some reports in extracting polysaccharides (Xie, Xie, Shen, Nie, Li and Wang, 2010).There was a decreasing trend in the polysaccharides yield from 300 to 400W of the extracting power. This tendency was in the agreement with other reports in extracting polysaccharides(Deng, Li & Yuan, 2006).The fitting equation was established, Y = 0.2859 +0.884 x_1 +1.031 x_2 -1.022 x_3 -0.244 x_4 +0.133 x_5 +0.379 x_6 , and by the statistical analysis of the polysaccharides yield equation, the experimental model was significant. The experimental datas were present in confidence band, and they were credible by the residual analysis.

The steepest climbing experiment results

According to the changing trend of the significant factors on polysaccharides extracted, the parameters of the extracting temperature, liquid-solid ratio increased gradually were designed, and the parameters of the power decreased gradually was designed, the largest polysaccharides yield response region can be found by this method. From Table 4, temperature 75 ℃, power 500W, liquid-solid ratio 75mL/g were the optimal parameters of the maximum polysaccharides yield, so these parameters were selected as the central composite design parameter.

The validation of the model

Table 5 showed the process variables and experimental data. The results of the analysis of variance, goodness of fit and the adequacy of the models were summarized. The

Table 4. Experimental design of steepest ascent and corresponding response

Number	Temperature（℃）	Power(W)	Liquid-solid ratio	Polysaccharides content（%）
1	65	600	65	8.15
2	75	500	75	8.22
3	85	400	85	8.10
4	95	300	95	8.12

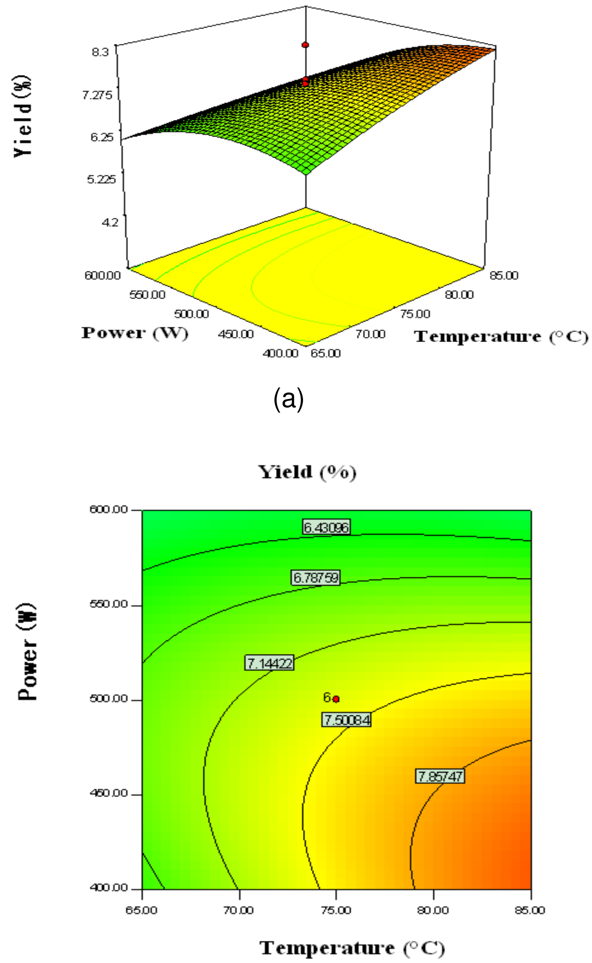
Table 5. Central Composite Design matrix (in coded level of three variables) and response values for the yield of *Stigma Maydis* polysaccharides.

Experiment	Independent variable coding			Model response value	
number	X_1	X_2	X_3	Experimental values（%）	Predictive value（%）
1	0	0	-1.68	6.15	6.84
2	0	0	0	8.3	7.39
3	0	-1.68	0	6.87	7.13
4	0	0	0	7.2	7.39
5	+1	-1	-1	8.6	8.13
6	0	0	0	6.98	7.39
7	+1	-1	+1	8.4	8.22
8	0	0	0	7.4	7.39
9	0	0	0	7.1	7.39
10	-1	+1	+1	6.72	6.53
11	-1	-1	-1	6.61	6.40
12	0	+1.68	0	4.21	4.88
13	-1	-1	+1	6.98	6.87
14	0	0	+1.68	7.51	7.74
15	-1.68	0	0	6.1	6.35
16	+1	+1	-1	6.32	5.79
17	0	0	0	7.5	7.39
18	+1	+1	+1	6.83	6.40
19	+1.68	0	0	7.05	7.70
20	-1	+1	-1	6.01	5.54

polysaccharides yield from 4.21% to 8.30%. To make fitting response equation with a spin and versatility, the number of the center point experiment was six. By applying multiple regression analysis on the experimental data, the predicted response Y for the polysaccharides yield can be obtained by the following second-order polynomial equation:

$$Y = 7.39 +0.4 x_1 -0.67 x_2 +0.27 x_3 -0.37 x_1 x_2 -0.096 x_1 x_3 +0.13 x_2 x_3 -0.13 x_1^2 -0.49 x_2^2 -0.035 x_3^2$$

,where x_1, x_2 ,and x_3 were the coded values of the test variables ,extracting temperature, extracting power, and the liquid-solid ratio, respectively. Statistical testing of the model was performed in the form of analysis of variance, which is required to test the significance and adequacy of the model. The date obtained by the software showed a good fit, the regression model P <0.05, it indicated that the model was significantly, and the possibility of F-value



(a)

(b)

Figure 1. Response surface plot of microwave power and temperature on polysaccharides yield

getting bigger by the noise was just 1.63%, and the models fitted well. Adeq precision used for the measurement signal to noise ratio, the ratio is greater than 4, showed that the model is expected.

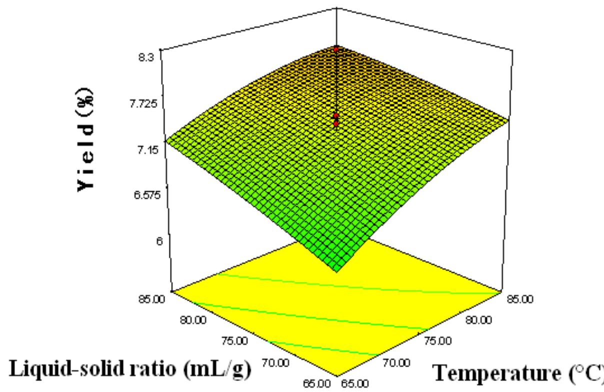
The regression model showed that the extracting temperature factor x_1 (P = 0.0360 <0.05) significant, power factor x_2 (P = 0.0022 <0.05) significant, liquid-solid ratio factor x_3 (P = 0.1319> 0.05) non-significant. The quadratic term of liquid-solid ratio x_3^2 (P = 0.0117 <0.05) significant, and the other quadratic and interactions terms were not significant. Estimated coefficients from three factors, the main effects relationship as follows: liquid-solid ratio> temperature> power.

Response surface analysis results

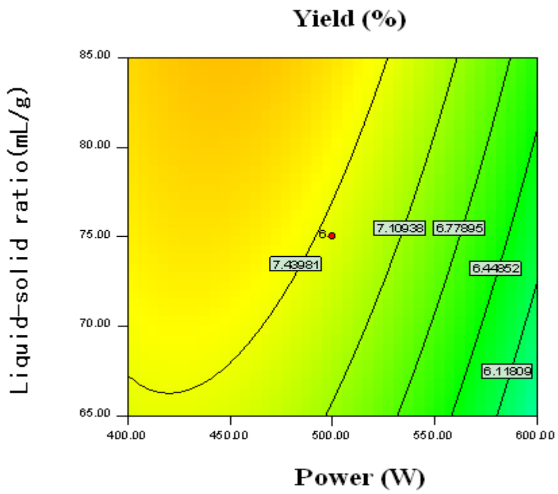
Response surfaces were plotted using Design-expert 7.0 software to study the effects of parameters and their interactions on polysaccharides yield. The effects of the ratio of liquid-solid, extraction temperature, and the power on polysaccharides yield are shown in Figure.1-3, respectively, the interaction effects of the factors on the responses is known clearly. It is very easy and convenient to understand the interactions between two variables and to locate their optimum ranges. In all presented figures, the other one factor was kept at level zero.

The effect of microwave power and temperature on the polysaccharides yield

Figure 1 displays the effect of the power and extraction temperature on the polysaccharides yield at the liquid-solid ratio 75mL/g.High yield was obtained at a low power and high extraction temperature. At the power of 400W, a temperature 85 ℃, liquid-solid ratio 75mL/g, the polysaccharides yield was 7.85%.



(a)



(b)

Figure 2. Response surface plot of microwave temperature and liquid-solid ratio on polysaccharides yield

The effect of temperature and liquid-solid ratio on the polysaccharides yield

The power was the major factor affecting the polysaccharides yield .Figure 2 presented the effect of extraction temperature and ratio of liquid-solid on polysaccharides yield at power 500W.the interaction between extraction temperature and liquid-solid gave rise to a higher yield at high temperature and high ratio of liquid-solid. At the temperature 85 ℃, power 500W, liquid-solid ratio 85mL/g, the polysaccharides yield was 8.21%.

The effect of power and liquid-solid on the polysaccharides yield

Figure 3 reveals the effect of the power and liquid-solid ratio on the polysaccharides yield at the extraction temperature 75 ℃.A high yield was obtained at a low power and high liquid-solid ratio. At a temperature of 75 ℃, power 400W, liquid-solid ratio of 85mL/g, the polysaccharides yield was 8.08%.

Comparison of microwave-directed and conventional extraction methods

In order to compare the results of microwave-directed with other traditional extraction methods, we performed all experiments using raw materials from the same batch, and the technology of extraction method is exactly the same as that given in the literature. Figure 4 shows that the microwave-directed extraction time is short. The microwave-directed for 15 min gave higher yield of polysaccharide than the other three traditional extractions

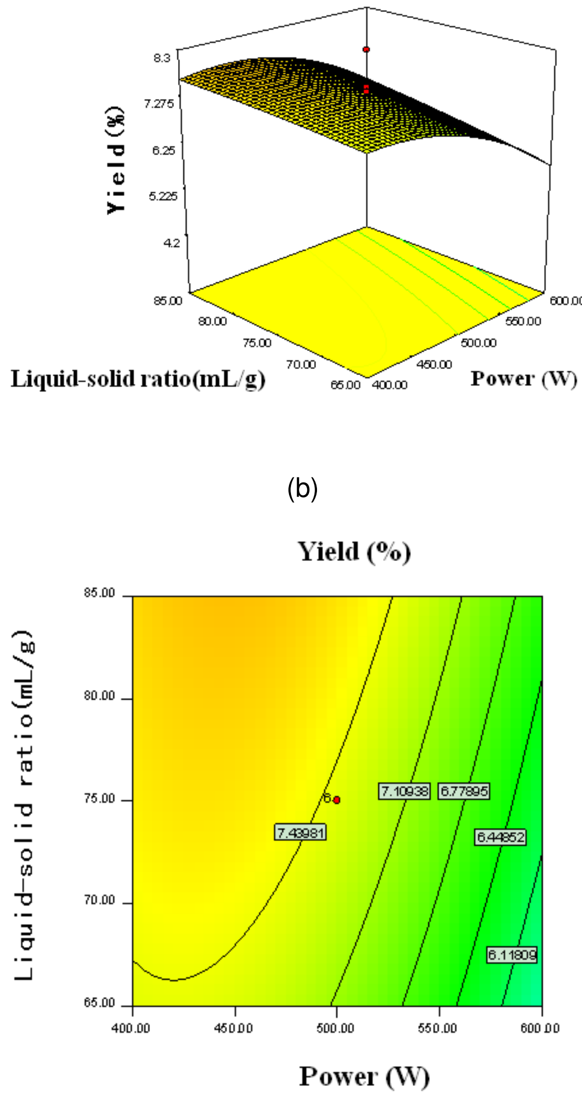


Figure 3. Response surface plot of microwave power and liquid-solid ratio on polysaccharides yield

for 20 、60 and 170min. The microwave- directed yield for 15 min is 2.02 times of the value obtained by enzyme extraction method(Wei, Lin & Zhu, 2007) for 170min, 3.12 times of the value obtained by ultrasonic -assistance extraction (Wei, Lin & Zhu, 2007)method for 20min, and 1.64 times of the value obtained by hot water extraction method.

CONCLUSIONS

In this paper, the significant factors of polysaccharides extracted from *Stigma Maydis* was screened by PB design of microwave-directed method, and the significant factors changing trends was predicted on the polysaccharides yield. The highest polysaccharides yield of process parameters had been established by the steepest climbing experiment, which was close to the optimal region. The optimal experimental region parameters were microwave power 500W, liquid-solid ratio 75mL/g, temperature 75 ℃. The optimized extraction condition of polysaccharides from *Stigma Maydis* was extraction temperature 85 ℃, extraction power 400W, and liquid-solid ratio 80mL/g for 15 minutes, respectively. Under the conditions, the polysaccharides yield of regression model was 8.93±0.43%, the real value was 9.36±0.22%, the relative deviation between the predicted valued and real value was less than 0.5%.More higher yields of the active polysaccharides obtained can support more active raw materials. Polysaccharides are

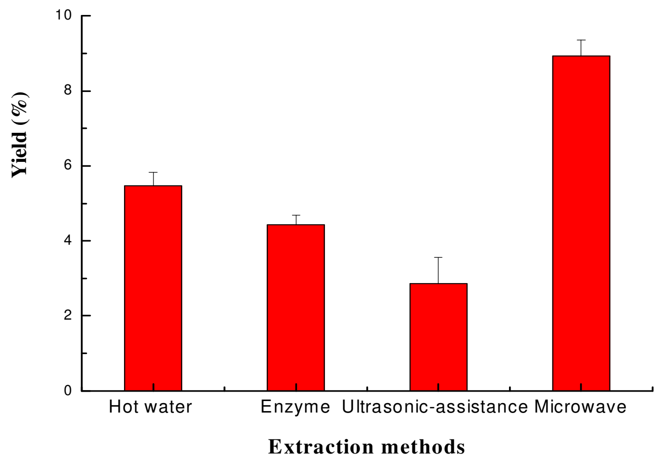


Figure 4. Compare of microwave-directed and conventional extraction methods

used as food ingredients in the food industry because of their functional properties of food systems, so Stigma Maydis polysaccharides further studies will provide a theoretical and research base to the rational use and development.

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