

Full Length Research Paper

Optimization of extraction technique of polysaccharides from pumpkin by response surface method

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A study on the extraction technique of pumpkin polysaccharides has been carried out. The effect of each factor such as the temperature of extraction, the time of extraction and the solvent-solid ratio on the extraction of the polysaccharides of pumpkin were investigated based on a three-level three-factor Box-Behnken design. The extraction technique parameters were optimized with response surface method. The result showed that the best technological conditions were that the extraction of temperature was 79.85, the extraction of time was 85.85 min and the solvent-solid ratio was 14.86: 1 by solving the regression equation with design-expert software. Under those conditions, the predicted value of the extraction ratio of polysaccharides of pumpkin was 12.4065%.

Keywords: Pumpkin polysaccharides, extraction, response surface method (RSM).

INTRODUCTION

Pumpkin (*Cucurbita pepo*) is annual sprawling herb belonging to the family of Cucurbitaceae (Hyun-II et al., 2006). It was planted in North America initially. It is also widely consumed in China. Pumpkin fruit are rich in carotene, pectin, mineral salts, polysaccharides, vitamins and other healthful substances (Marianna et al., 2009; Wang and Xu, 2004). Pumpkin has received considerable attention in recent years because of the nutritional and health protective value of the fruit.

Pumpkin polysaccharides (PP) are composed of galactose, glucose, arabinose, xylose and glucuronic acid and are water insoluble and organic solvents soluble macromolecular compounds with important biological function. PP has the biological effects of detoxification, anti-oxidation, reducing blood pressure, reducing blood lipids (Kong et al., 2000), lowering cholesterol levels (Wang and Xu, 2004; Yong et al., 2006), promote the

biosynthesis of nucleic acids and proteins, control cell division and differentiation, regulating cell growth and aging (Zhang et al., 2002), especially for the treatment of diabetes (Bai and Zhang, 2006).

Currently, the extraction methods of PP mainly include hot water extraction, ultrasonic extraction and enzyme extraction (Xu et al., 2006). Ultrasonic extraction technology has been widely used in the extraction of plant active ingredients, the main mechanism of ultrasonic extraction is due to cavitation generated by ultrasound (Zhang, 2006). The advantage is to greatly increase the extraction yield of active ingredients, reduce the extraction time and avoid the destruction of the active ingredients (Guo, 2003). But it is not suitable for practical application because of its high cost. Enzyme extraction hydrolyze the cellulose, pectin and crude protein, and break down the cell walls. It is a more advanced and effective extraction method (Xu et al., 2006).

Water extraction is the traditional method of polysaccharide. This method is simple. But the parameters of its extraction only remain in the single factor test, ignore the process of PP interaction between

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the factors. The extraction of PP from pumpkin fruit may be affected by various factors, such as the temperature of extraction, the time of extraction and the solvent-solid ratio. When many factors and interactions affect the desired response, the response surface method (RSM) is an effective tool for finding their optimal values (Triveni et al., 2001). RSM is a statistical-mathematical method which uses quantitative data in an experimental design to determine and to solve, multivariable equations in order to optimize processes or products (Giovanni 1983). RSM has been very popular for optimization studies in recent years. Nowadays, RSM is widely applied for different purposes in chemical and biochemical processes. These processes are affected by numerous variables and it is necessary to select those that have major effects as well as to identify their levels (Draginja et al., 2008).

The aim of the present work is to study the effects of the temperature of extraction, the time of extraction and the solvent-solid ratio on the PP as well as to optimize extraction conditions by using the RSM.

MATERIALS AND METHODS

Pumpkin was provided by the "jiutouya supermarket", Pingdingshan City, Henan Province, China.

Preparation of pumpkin powder

The pumpkin was cut and the seeds were removed. The rest were dried at 60°C and ground in a grinder. The pumpkin powder was kept at the temperature of 4°C.

Extraction of water-soluble polysaccharides from pumpkin

One gram of pumpkin powder was weighed for different optimization tests including extraction temperature (20, 40, 60 and 80°C), extraction time (30, 60, 90 and 120 min), solid-liquid ratio (1:10, 1:20, 1:30 and 1:40 g/ml). Crude water-soluble polysaccharides were extracted with pure hot water and precipitated with 95% ethanol, yielding the pumpkin polysaccharides. The precipitation was dried to calculate the yield.

Experimental design and statistical analysis

The effect of the three variables at three variation levels on PP extraction ratio was studied to determine the optimum combination of the variables, using the central-composite experimental (Box-Behnken) design for the RSM (Alessandra et al., 2009; Rodrigues et al., 1993). The model proposed for the response Y (extraction RATIO) is given below:

$$Y=b_0+b_1A+b_2B+b_3C+b_4A^2+b_5B^2+b_6C^2+b_7AB+b_8AC+b_9BC \quad (1)$$

where b_0 was offset term, b_1 , b_2 and b_3 were related to the linear effect terms, b_4 , b_5 and b_6 were connected to the quadratic effects and b_7 , b_8 and b_9 were associated with the interaction effects.

The variables and their ranges were as follows:

A: the temperature of extraction (40 to 80°C); B: the time of extraction (60 to 120 min) and C: the solvent-solid ratio (10:1 to 30:1). The adequacy of the polynomial model was expressed by the multiple coefficient of determination, R^2 . The significance of each coefficient was determined by using F value and P value. Moreover, in order to deduce workable optimum conditions, a graphical technique was used by fixing one variable at a predetermined optimum condition. The optimum condition was verified by conducting experiments under these conditions. The response was monitored and the results were compared with the model predictions. Statistical analysis was performed by using design-export.

RESULTS AND DISCUSSION

Results of RSM

The regression equation representing the relationship between PP extraction ratio and the test variables derived from RSM were as follows:

$$Y=11.17+1.16A-0.39B-0.83C-0.42AB-1.65AC+0.95BC-0.47A^2-0.92B^2-2.85C^2 \quad (2)$$

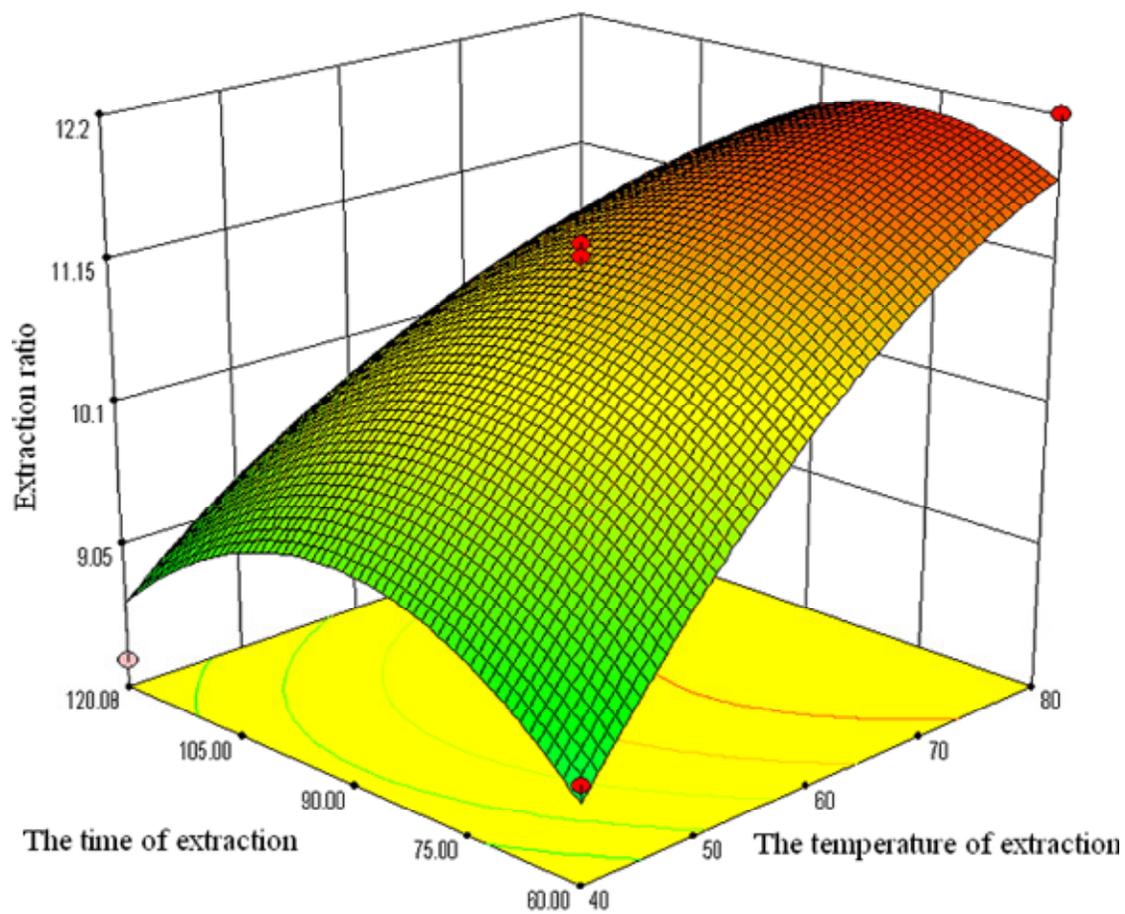
Y is a coded fitting equation. When the p value is less than 0.05, it shows that this item is significant, on the other hand, when the p value is less than 0.001, it shows that the item is highly significant and that means this item had a greater influence than other variables (Cai et al., 2007). F and p values, which determine the significance of each coefficient, were presented in (Table 1). The corresponding variables will be more significant if the absolute F value becomes larger and p value becomes smaller. It can be seen that the most significant variable was linear the temperature of extraction (A), followed by the time of extraction (B^2). The F values for A and B^2 are: 11.84 and 11.93, respectively, but the p values which seem more significant are: 0.0176 and 0.0153, respectively. According F and p values the terms of b_2 , b_4 , b_6 , b_7 and b_8 did not have the statistical significance.

The multiple coefficient of correlation ($R = 0.9600$) and the total determination coefficient ($R^2 = 0.9340$) indicate high agreement between experimental and predicted values of the PP extraction ratio. The 3D surface plots were drawn to illustrate the main and interactive effects of the independent variables on the dependent one. The response surface, whose coefficient are shown in (Figure 1). One of the variables kept at optimum level while the remaining two variables were changed within experimental range. In general, an analysis of the response surface indicated that a complex interaction between the variables exists. The effect of temperature and time on extraction ratio of PP is presented in (Figure 1a). The temperature of the extraction has linear effect on PP extraction ratio, whereas the time of extraction has quadratic effect.

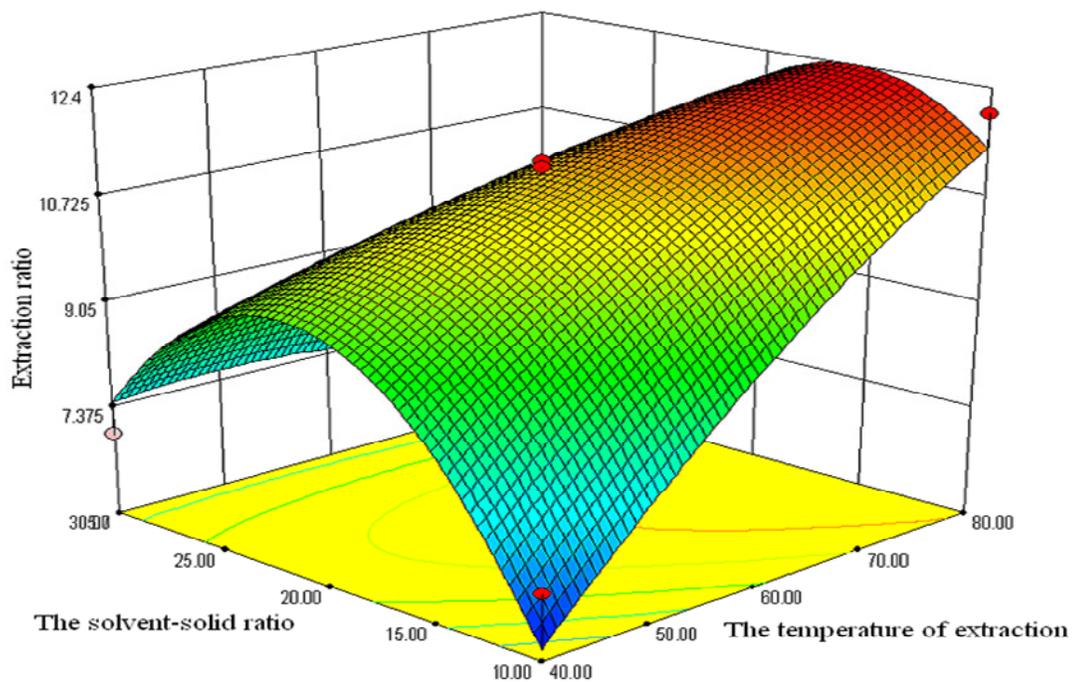
The influence of temperature and solvent-solid ratio is shown in (Figure 1b). The extraction ratio is increasing

Table 1. Significance of regression equation coefficients for the extraction ratio.

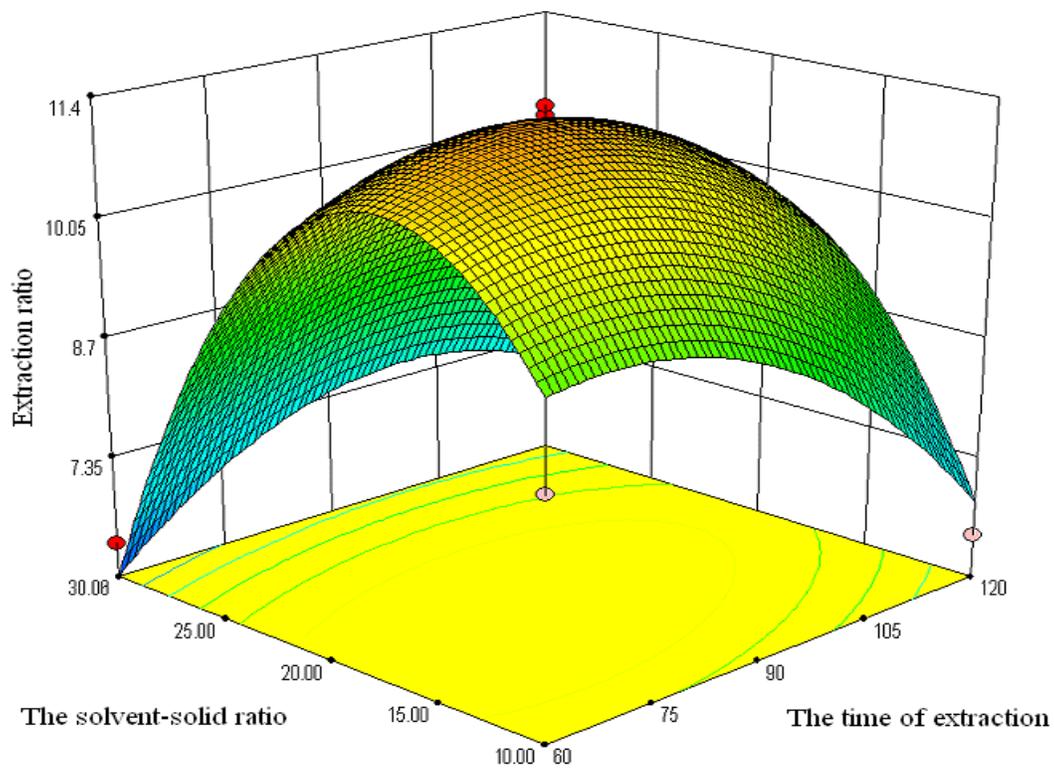
Variables	Regression coefficients	Standard error	Computed F value	Significance level (P value/)
b ₀	11.17	0.55	7.86	0.0176
Linear				
b ₁	1.16	0.34	11.84	0.0184
b ₂	-0.39	0.34	1.32	0.3032
b ₃	-0.83	0.34	5.96	0.0585
Quadratic				
b ₄	-0.42	0.48	0.79	0.4144
b ₅	-1.65	0.48	11.93	0.0182
b ₆	0.95	0.48	3.95	0.1034
Interaction				
b ₇	-0.47	0.50	0.90	0.3872
b ₈	-0.92	0.50	3.43	0.1232
b ₉	-2.85	0.50	32.76	0.0023
R	0.9600			
R ²	0.9340			



a



b



c

Figure 1. 3D graphics surface optimization of the extraction technique of PP (a) temperature and time, (b) temperature and solvent-solid ratio and (c) time and solvent-solid ratio.

with increasing temperature, temperature has linear effects and solvent-solid ratio has little effects. Influence of time and solvent-solid ratio is shown in (Figure 1c). These results have shown that the response surface had a maximum point. The maximum extraction ratio of PP (12.4065%) was obtained when the temperature is 79.85°C, the time is 85.85 min and solvent-solid ratio is 14.86:1.

Verification of results

In order to examine whether the equation gained from RSM could fit the relationship between extraction ratio and each variable well, confirmatory experiment was carried out. Considering the feasibility of the actual operation, solvent-solid ratio was adjusted to 15:1 and time was 86 min. The extraction ratio gained from the revised craft reached 12.3581%, with an error of 0.39% comparing to optimal extraction ratio, which showed that the technology of PP extraction obtained from RSM was accurate and reliable Valeria (2007).

Conclusions

The present study has shown that the extraction ratio of the PP was optimized by using Statistical software. The three independent variables, involved in the optimization, were the temperature of extraction, the solvent-solid ratio and the time of extraction. The RSM result indicated that the variable with the largest effect was the temperature of extraction. That was followed by the time of extraction and the solvent-solid ratio. Moreover, the optimal PP yield of 0.124065 g from 1 g pumpkin was obtained.

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