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Short communication

Enzyme assisted extraction of polysaccharides from the fruit of *Cornus officinalis*

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ABSTRACT

Process of enzyme assisted extraction (EAE) of polysaccharides from *Cornus officinalis* was optimized by response surface methodology (RSM). The influence of four different factors on the yield of *C. officinalis* polysaccharides (COP) was studied. Results showed that the optimal conditions were compound enzyme amount of 2.15%, extraction pH of 4.2, extraction temperature of 55 °C and extraction time of 97 min. Under these conditions, the COP yield was 9.29 ± 0.31%, which was well in agreement with the value predicted by the model. The three methods, EAE, hot water extraction (HWE), ultrasound-assisted extraction (UAE) for extracting COP by RSM were further compared. Results showed that EAE had the largest yield of polysaccharides with lower equipment cost.

1. Introduction

Cornus officinalis has been used as a Chinese medicine for more than 2000 years for its tonic, diuretic and analgesic effects (Cai-ying, Liang, Hui feng, & Xue-ying, 2007). The fruit of *C. officinalis* has many bioactivities such as anti-microbial, anti-oxidant, anti-aging, and immunoregulatory activities (Cai-ying & Liang, 2007) and the main bioactive ingredient may be polysaccharides (Yunxiang, Guanye, & Xia, 2009).

The extraction methods of polysaccharides commonly include hot water extraction (HWE), enzyme assisted extraction (EAE) and ultrasound-assisted extraction (UAE) (Yin, You, & Jiang, 2011). EAE is an emerging skill in the food industry since it provides many advantages such as high extraction yield, low energy consumption, high repeatability and simplified manipulation (Fan et al., 2011; Zhang, Jia, Liu, Wu, & Ran, 2011). Response surface methodology (RSM) is an efficient statistical method for optimizing complicated processes because it allows more efficient and easier arrangement and interpretation of experiments compared to other methods (Gan & Latiff, 2011). Moreover, it is less laborious and time-consuming than other methods (Gan, Manaf, & Latiff, 2010).

In our present paper, three methods (HWE, EAE and UAE) were compared by the yields of *C. officinalis* polysaccharides (COP) by RSM, and basic physicochemical characteristics of polysaccharides by three methods were compared.

2. Materials and methods

2.1. Materials and equipments

The fruits of the *C. officinalis* were purchased from drugstore. Samples were dried at 60 °C for 12 h and grinded. Ten grams of dried samples was firstly degreased with petroleum ether (60–90 °C) and pretreated with 80% ethanol twice, subsequently, the pretreated samples were separated from the solvent by centrifugation and were dried until its weight was constant. Ultrasonic Cell Disruption System (JY92-II, Xinzhi Bio-technology and Science Inc.) and Thermostat water bath (HH-2, Jintan Jingda Instrument Factory) was used for polysaccharides extraction. Cellulase and papain were acquired from Sinopharm Reagent Co., Ltd. Pectinase were obtained from TianJin Enzyme Co., Ltd. All other reagents were analytically pure. All the experiments data were an average of three parallel replicates.

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Table 1
Response surface design and polysaccharides yield.

Run	X_1 (%)	X_2	X_3 (°C)	X_4 (min)	Y (%)
1	1.70	3.70	40.00	60.00	1.87
2	1.70	3.70	40.00	120.00	5.52
3	1.70	3.70	60.00	60.00	2.17
4	1.70	3.70	60.00	120.00	7.86
5	1.70	4.30	40.00	60.00	7.38
6	1.70	4.30	40.00	120.00	7.77
7	1.70	4.30	60.00	60.00	7.98
8	1.70	4.30	60.00	120.00	8.52
9	2.30	3.70	40.00	60.00	2.46
10	2.30	3.70	40.00	120.00	5.48
11	2.30	3.70	60.00	60.00	5.51
12	2.30	3.70	60.00	120.00	8.26
13	2.30	4.30	40.00	60.00	7.86
14	2.30	4.30	40.00	120.00	8.37
15	2.30	4.30	60.00	60.00	8.73
16	2.30	4.30	60.00	120.00	8.48
17	1.40	4.00	50.00	90.00	7.82
18	2.60	4.00	50.00	90.00	8.41
19	2.00	3.40	50.00	90.00	3.36
20	2.00	4.60	50.00	90.00	8.58
21	2.00	4.00	30.00	90.00	7.26
22	2.00	4.00	70.00	90.00	8.01
23	2.00	4.00	50.00	30.00	5.13
24	2.00	4.00	50.00	150.00	8.36
25	2.00	4.00	50.00	90.00	8.81
26	2.00	4.00	50.00	90.00	8.42
27	2.00	4.00	50.00	90.00	8.67

2.2. Extraction methods

2.2.1. HWE of polysaccharides

The pretreated sample (5.0 g) was extracted by certain volume pure water at specific temperature for certain time. The suspension was centrifuged and the insoluble residue was handled again for two times as mentioned above. The combined supernatant was concentrated by evaporator at 50 °C. The supernatant was deposited by the addition of anhydrous ethanol to a final concentration of 80% and the precipitates were obtained by centrifugation. After being washed three times with anhydrous ethanol, the precipitate was air-dried at 50 °C until its weight was constant.

2.2.2. EAE of polysaccharides

The pretreated sample (5.0 g) was soaked in 200 ml sodium hydroxide–citric acid–chlorhydric acid buffer and then was extracted with compound enzyme (the proportion of cellulase:pectinase:papain was 1:1:1) at fixed pH and time while the water bath was maintained at a given temperature during the extraction process. RSM extractions were implemented on the basis of one-factor experimental design (Table 1). The following procedure was consulted for Section 2.2.1.

2.2.3. UAE of polysaccharides

The pretreated sample (1.0 g) was extracted with pure water in a beaker which was put in the Ultrasonic Cell Disruption System and extracted for various time at varied ultrasonic power. Ice bath was applied to guarantee that the temperature of the extraction solution was below 60 °C during extraction process. The following procedure was consulted for Section 2.2.1.

2.3. Analytical methods

The content of the polysaccharides was determined by phenol–sulfuric method (Masuko et al., 2005). The yield of COP (%) was computed as follow:

$$\text{The yield of COP}\,(\%) = \frac{\text{the polysaccharides content of extraction(g)}}{\text{weight of cornus officnalis powder(g)}} \times 100\% \qquad (1)$$

2.4. Experimental design and analysis

The extraction parameters were optimized by RSM. The behavior of the polysaccharide extraction was explained by second degree polynomial model:

$$y = \beta_{k0} + \sum_{i=1}^{4} \beta_{ki} X_i + \sum_{i=1}^{4} \beta_{kii} X_i^{2} + \sum_{i<j=2}^{4} \beta_{kij} X_i X_j \qquad (2)$$

y is the response value,β_{k0} is an constants, β_{ki}, β_{kii} and β_{kij} are the coefficients of the linear, quadratic and interactive terms, respectively. And accordingly X_i and X_j represent the coded independent variables. According to the analysis of variance (ANOVA), the effect and regression coefficients of individual linear, quadratic and interaction terms were measured. The regression coefficients were subsequently applied to make calculation to generate dimensional and contour maps from the regression equation.

3. Results and discussion

3.1. Optimization of the EAE conditions

The values of regression coefficients were computed, predicted response Y for the yield of COP could be expressed by the following polynomial model according to coded values:

$$\begin{aligned} Y = {} & 8.63333 + 0.60500X_1 + 3.03333X_2 + 1.02500X_3 + 1.89667X_4 \\ & - 0.46000X_1^2 - 1.53250X_2^2 - 0.70000X_3^2 - 1.14500X_4^2 \\ & - 0.31250X_1X_2 + 0.35250X_1X_3 - 0.53000X_1X_4 - 0.76750X_2X_3 \\ & - 1.74000X_2X_4 + 0.14500X_3X_4 \end{aligned} \qquad (3)$$

where Y is the yield of COP (%), X_1, X_2, X_3 and X_4 are the coded variables for compound enzyme amount, extraction pH, extraction temperature and extraction time, respectively.

The low p-value ($p = 0.001867$) indicated that this model was highly statistical significance. ANOVA (data not show) indicated that the linear coefficients (X_1, X_2, X_3, X_4), quadratic coefficients ($X_2{}^2$, $X_2{}^2$, $X_3{}^2$, $X_4{}^2$) and cross product coefficients ($X_1 \times X_4$, $X_2 \times X_3$, $X_2 \times X_4$) were significant, with small p-values ($p < 0.04$). The other coefficients ($X_1 \times X_2$, $X_1 \times X_3$, $X_3 \times X_4$) were insignificant ($p > 0.05$). Results indicated that the compound enzyme amount, extraction time, temperature and pH were all significantly correlated with the yield of COP. The three-dimensional and contour maps were made by Eq. (3) to predict the relationships between independent factor and dependent factors. The results of the yield of COP which affected by compound enzyme amount, extraction temperature, time and pH are shown in Figs. 1 and 2. The optimal values of the factors for obtaining yield of COP (9.46%) can be predicted as follows: compound enzyme amount (2.146%); extraction pH (4.21); extraction temperature (55.02 °C) and extraction time (96.79 min). Among four factors, pH was the most significant variable that affects the yield of COP, followed by extraction time, extraction temperature and compound enzyme amount according to the regression coefficients significance of the equation and gradient of slope in the 3D response surface map (Figs. 1 and 2).

The optimum conditions can be modified as follows: compound enzyme amount (2.15%), extraction pH (4.2), extraction temperature (55 °C) and extraction time (97 min). To validate the adequacy of the model equations, a verification experiment is carried out

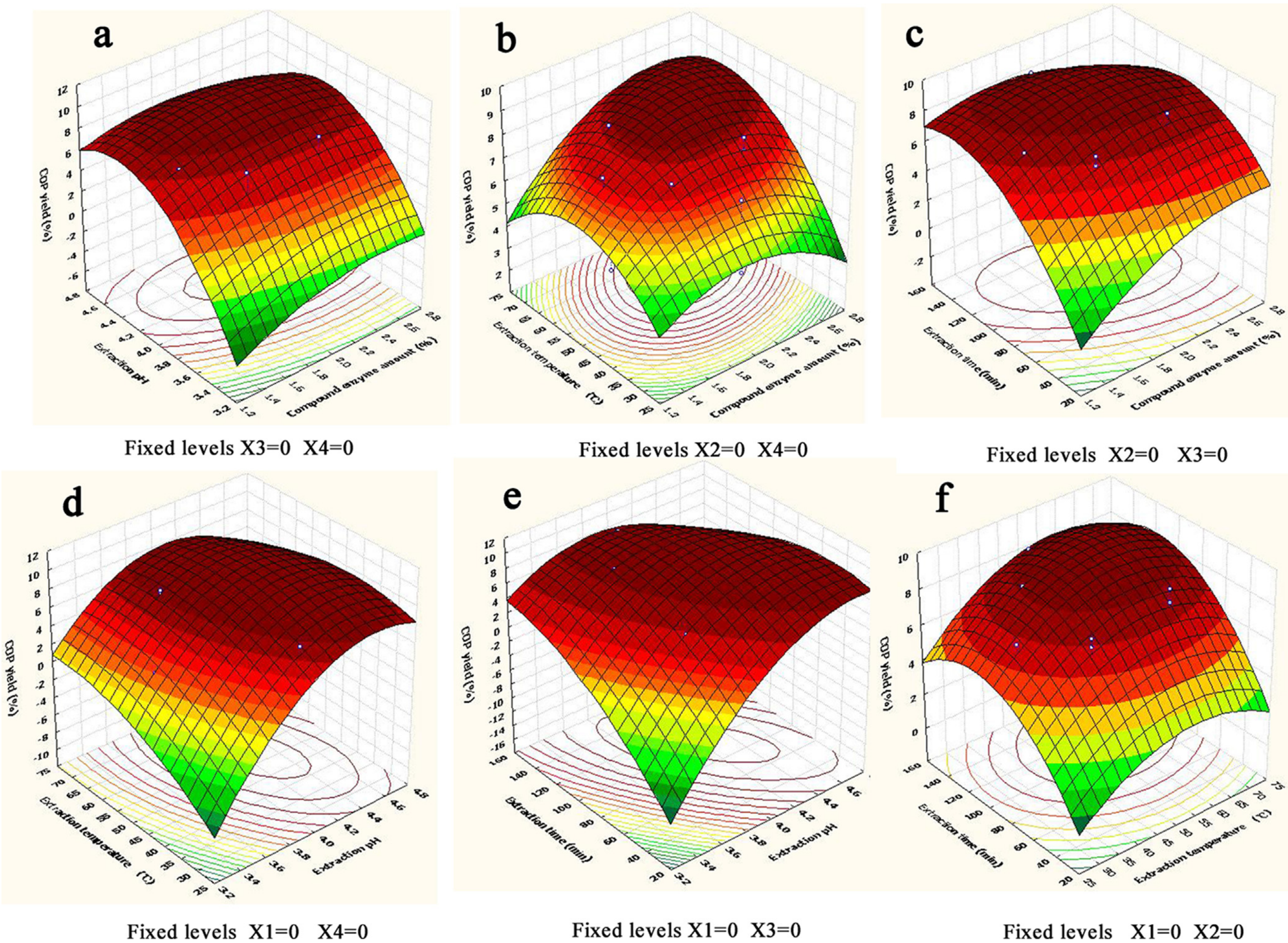

Fig. 1. Response surface (3D) showing the effect of the compound enzyme amount, extraction temperatures, pH and time on the response *Y*.

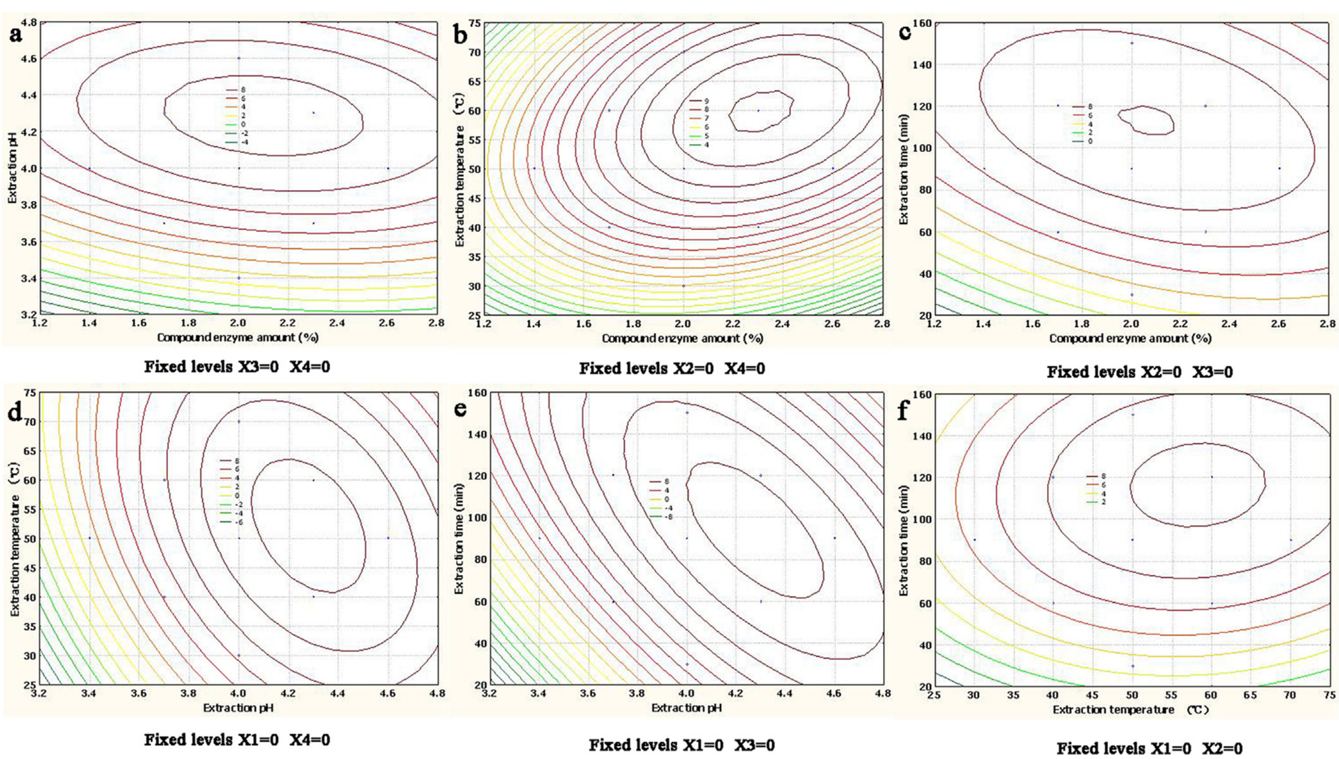

Fig. 2. Contour plots showing the effect of the compound enzyme amount, extraction temperatures, pH and time on the response *Y*.

Table 2
Comparison of the yields and basic physicochemical characteristics of polysaccharides of the three methods.

Method	EAE	UAE	HWE
Compound enzyme amount (%)	2.15		
Extraction pH	4.2		
Extraction time (min)	97	3.5	97
Extraction temperature (°C)	55	Icebath	97
Ratio of water to raw material (ml/g)		60.6	24.1
Ultrasonic power (W)		430	
Equipment costs	Low	High	Low
Operator skill	Low	Medium	Low
COP yield (%)			
Predicted	9.46	8.89	8.68
Experimental	9.29 ± 0.31	8.95 ± 0.26	8.53 ± 0.33

under optimum conditions as mentioned above. Under optimal conditions, the yield of COP is 9.29 ± 0.31% (n = 3), which was well-matched with the predicted value obtained from the model (data not show). The result demonstrates the validation of the model.

3.2. Comparison of EAE, UAE and HWE

RSM were similarly applied for optimizing the extraction process of UAE and HWE. The COP yield of EAE, UAE and HWE were compared in Table 2. The yield of COP by EAE was higher than that of UAE and HWE. The COP yield of UAE extracted for 3.5 min was higher than that of HWE extracted for 97 min. This indicated that UAE had higher extraction efficiency with shorter time. Compared with HWE, EAE had lower extraction temperature with higher COP yield. UAE could efficiently reduce extraction time under low frequency, but it requires higher equipment cost and operator skill. The price of ultrasonic instrument (about 10,000 $) is much higher than that of water bath (about 100 $). These results demonstrated that EAE was a promising extraction method that offers higher yield and lower equipment cost.

4. Conclusion

EAE was employed to extract polysaccharides from the fruit of *C. officinalis*. RSM was used to estimate and optimize experimental variables based on one-factor experimental design. Results showed that an optimum yield of COP could be obtained when conditions were set as follows: compound enzyme amount of 2.15%, extraction pH of 4.2, extraction temperature of 55 °C and extraction time of 97 min. Under optimum conditions, the maximum COP yield was 9.29 ± 0.31%, which was in accordance with the predicted value. UAE and EAE were better than HWE in extracting COP. However, EAE showed higher yield of COP and lower equipment costs than UAE.

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