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Research Article

Optimization of Ultrasound-assisted Extraction of *Lentinula Edodes* Polysaccharides Using Response Surface Methodology

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Abstract: In this study, an ultrasound-assisted procedure for the extraction of *Lentinula edodes* polysaccharides was established. Response Surface Methodology (RSM) was used to optimize the ultrasound-assisted extraction parameters (extraction temperature (A), extraction time (B) and ratio of water to raw material (C)) for enhancing the forward extraction efficiency of *Lentinula edodes* polysaccharides by implementing a three-level, three-variable Box-Behnken experimental design. The independent variable with the largest effect on response was A (extraction temperature), followed by C (ratio of water to raw material) and B (extraction time). The optimum extraction conditions were found to be extraction temperature 76.6°C, extraction time 88.8 min and ratio of water to raw material 58.1 (mL/g). Under these conditions, theory of extraction yield of 3.83%.

Keywords: *Lentinula edodes* polysaccharides, Response Surface Methodology (RSM), ultrasonic-assisted extraction

INTRODUCTION

The white rot basidiomycete *Lentinula edodes* (Berk.) Pegler (the edible shiitake mushroom) is the most widely cultivated mushroom in East Asia and its cultivation is regarded as the largest bioconversion process utilizing wood (Buswell *et al*., 1995). Shiitake is the second most popular in the world (Sugui *et al*., 2003). The polysaccharide lentinan which has demonstrated high immunopotentiating activities (Kupfahl *et al*., 2006), antitumor activity (Maruyama *et al*., 2006), antibacterial, antifungal and anti diabetic activities (Markova *et al*., 2003), among others. Polysaccharides are the best known and most potent mushroom-derived substances with antitumor and immunomodulating properties. It exists as a structural component of fungal cell wall. Fungal cell wall is composed of two major types of polysaccharides: one is a rigid fibrillar of chitin (or cellulose), the other one is a matrix-like β-glucan, α-glucan and glycoproteins (Ruiz-Herrera, 1956) Selection of an extraction method depends on the cell wall structure. Hot water extraction has been a popular approach. The extraction with hot water yielded water-soluble polysaccharides and the extraction with alkali solution yielded the water-insoluble ones. The extraction method can be varied based on the structure and water-solubility of polysaccharides but the basic rule is to break the cell wall from outer layer to the Inner layer with mild-to-strong extraction pH and temperature. However, the simple hot water extraction to extract the time long, impurities and can only extract extracellular polysaccharide, dilute acid extraction easy cause glycosidic bond rupture, dilute alkali extraction to extract the polysaccharide in more impurities, easily causes hydrolysis of the polysaccharide. Ultrasonic extraction technology has been used to extract high quality bioactive polysaccharide, using the ultrasonic assisted hot water extraction polysaccharides can effectively shorten the extraction time, increase extraction yield (Wang *et al*., 2012).

Response surface methodology is a collection of statistical and mathematical techniques useful for developing, improving and optimizing process. The main advantage of Response surface methodology is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions (Chen *et al*., 2010). In this study, on the base of single factor experiment, optimized by an response surface methodology.

MATERIALS AND METHODS

Plant material: The samples of dry *Lentinula edodes* were collected in Liaoning Province (2013), in China. All the collected dry mushroom were dried at 50°C remove containing water, according to constant weight and loaded valve bag. After drying the raw material of disintegrator (Teste instrument Co. Ltd. Tianjin, China) grinding into fine powder then with a sieve.

The establishment of the standard curve: Accurately according to the standard glucose 20 in 500 mL volumetric flask and add water to the scale,

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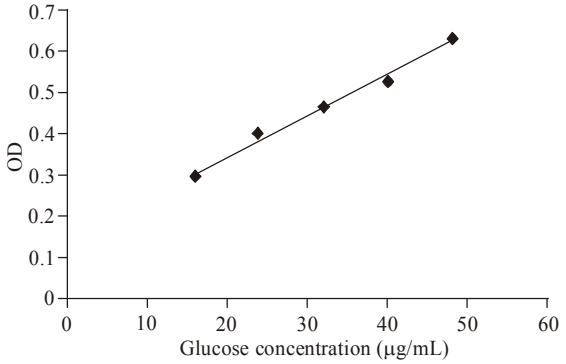

Fig. 1: Glucose standard curve

Table 1: Factors and levels of the response surface optimization design

Factor	Low	Center	High
Extraction temperature (°C, A)	-1 (70)	0 (80)	1 (90)
Extraction time (min B)	-1 (60)	0 (90)	1 (120)
Ratio of water to raw material (C)	-1 (50)	0 (60)	1 (70)

respectively, from 0.4, 0.6, 0.8, 1.0 and 1.2 mL, filling water to 2.0 mL. Add 6% phenol 1.0 and 5.0 mL of sulfuric acid and still standing for 30 min after 490 nm absorbance measurement, with 2.0 mL distilled water according to the same color as the blank, operation with horizontal axis as the glucose concentration, Y coordinate for the absorbance value, draw the standard curve. Standard curve as shown in Fig. 1.

Extraction of *Lentinula edodes* polysaccharides and determination of the yield: The pretreated dry powder (1.0 g) with distilled water. The single factor experiment was performed in a designed extraction temperature (range from 50 to 90°C), extraction time (range from 60 to 180 min) and ratio of water to material (range from 40 to 80 mL/g). After hot water extraction of extract in the ultrasonic cleaners (KQ5200DB, Kunshan ultrasonic instrument Co., LTD., Jiangsu, China) 80 W oscillation 13 min. The supernatant was separated from insoluble residue by circulating multi-purpose vacuum pump (SHB-Ⅲ, Zhengzhou Great Wall industry trade Co. Ltd., Henan, China) and concentrated to 1/3 of the original volume with rotary evaporator (N-1001, Ailang instrument Co., Ltd., Shanghai, China) and then add three times the volume of 95% vol ethanol solution, then kept at 4°C overnight and the precipitates were collected by centrifugation and stoving to get the *Lentinula edodes* polysaccharides.

According to take different source extraction of *Lentinula edodes* polysaccharides 5 mg, water soluble, according to the preparation of standard curve method for determining absorbency. The yield (%) of *Lentinula edodes* polysaccharides was calculated using the following equation:

$$Yield = (C \times 100 \times m) / (1000 \times W \times M)$$

where,

C = Measured sample solution mass concentration of glucose (mg/mL)
M = Said to take quality of *Lentinula edodes*, g
W = The quality of the product, mg
Yield = Polysaccharide extraction yield, %
M = Alcohol polysaccharide quality resulting from the sink and the unit is g

The response surface optimization design of experiment: On the basis of single factor experiment, with extraction temperature (A), extraction time (B), ratio of water to raw material (C) than as independent variable, in the center of the three factor and three level Box-Benhnken combination experimental design, factor level table as shown in Table 1. According to regression analysis to determine the response of the various process conditions factor, *Lentinula edodes* extraction Yield (Y) as the response value to do the response surface.

Statistical analysis: The data of seed phenotypic traits were input into computer by a trained worker and analyzed by statistic software STATA 8.0, EXCEL and SPSS 13.

DALP amplified fragments, with the same mobility according to the molecular weight, were scored by eyes for the presence (1), negative (0), or absence (.) of homologous bands. The resulting presence/negative/ absence data matrices of the DALP phenotypes were analyzed using Popgene version 1.31 to estimate genetic diversity parameters: Percentage of Polymorphic Bands (PPB), Shannon's index of phenotypic diversity (I), mean observed number of alleles (Na), mean effective number of alleles (Ne), Nei's gene diversity (H), total gene diversity (Ht), gene diversity within population (Hs), the coefficient of gene differentiation (Gst) and the level of gene flow (Nm). To examine the genetic relationship at the species and population level, dendrograms were constructed by an Un-weighted Pair Group Method of cluster Analysis using arithmetic averages (UPGMA).

RESULTS

The standard curve of glucose: As shown in Fig. 1, with glucose as the independent variable, the optical density value of the dependent variable, the polysaccharide concentration C and absorbance value. OD values of linear regression curve, the equation is as follows:

$$Y = 0.0095X + 0.1424, R^2 = 0.9905$$

Show good linear relationship.

Effect of extraction temperature on the yield of *Lentinula edodes* polysaccharides: In this study, extraction temperature was set at 50, 60, 70, 80 and 90°C, respectively, to investigate its effect on the

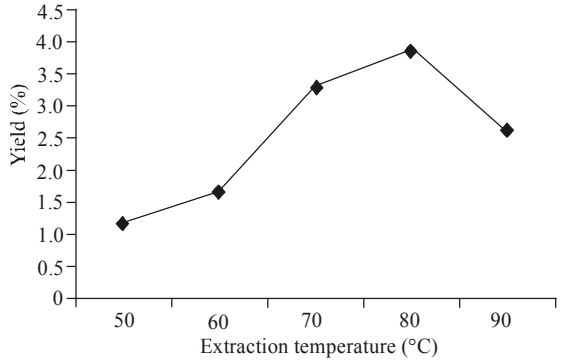

Fig. 2: Effects of different extraction temperature parameters

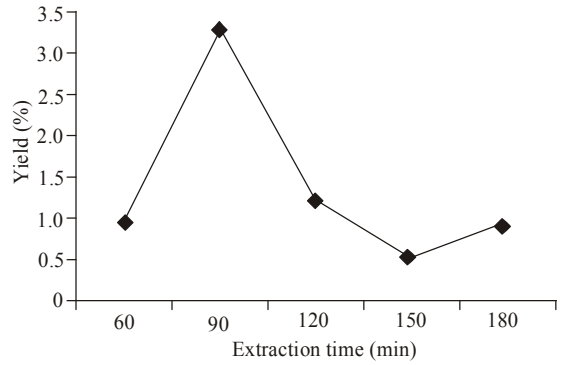

Fig. 3: Effects of different extraction time parameters

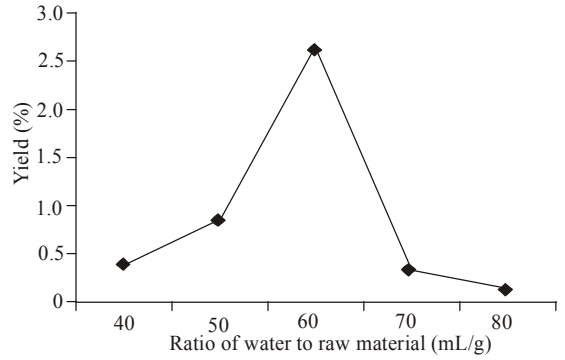

Fig. 4: Effects of different ratio of water to material parameters

extraction yield of polysaccharides when other parameters were set as follows: ratio of water to material 60 mL/g, extraction time 120 min. As can be seen in Fig. 2 the extraction yield of polysaccharides increased quickly within the initial 80 min and reached the maximum extraction yield is 3.82%. After 80°C, polysaccharide yield decreased with increasing temperature, may be because the temperature is high, the ultrasonic cavitation and high temperature under the dual function of polysaccharide degradation, thus lowered the rate. Therefore, this study the extraction of lentinan selected a temperature of 80°C is more appropriate.

Table 2: Experiment of extraction of polysaccharides from *Lentinula edodes*

Number	A	B	C	Y
1	0	-1	1	0.86
2	1	0	1	1.22
3	1	-1	0	0.66
4	0	0	0	3.83
5	1	1	0	0.88
6	0	1	-1	1.01
7	-1	-1	0	1.29
8	0	0	0	3.84
9	1	0	-1	0.29
10	-1	0	-1	0.38
11	0	-1	-1	0.19
12	0	0	-1	3.83
13	0	1	1	1.34
14	-1	0	1	2.57
15	-1	1	0	3.37
16	0	0	0	3.83
17	0	0	0	3.82

Effect of extraction time on the yield of *Lentinula edodes* polysaccharides: Effect of extraction time (60-180 min) on the extraction yield of *Lentinula edodes* polysaccharides was investigated and other experimental parameters were extraction time 80°C, ratio of water to material 60 mL/g. The results (Fig. 3) indicated that the yield significantly increased when the time increased from 60 to 90 min, this is because, under the action of ultrasonic wave and hot water, the cells are destroyed completely, polysaccharide material leaching in full. When extracting time more than 90 min, coarse polysaccharide yield showed a trend of sharp decline in, the reason may be that much at 60 and 120 min leaching quantity is little, the polysaccharide yield rose slightly after 150 min, the polysaccharide extraction quantity increase, this is because the reducibility of lentinan compares strong, easy to oxidation by oxygen in the air, because the time is too long to polysaccharide compound oxide, leading to higher extraction yield. Extracted molecules and the improved mass transfer at higher temperature.

Effect of ratio of water to material on the yield of *Lentinula edodes* polysaccharides: In this study, ratio of water to material was set at 40, 50, 60, 70 and 80 mL/g, respectively to investigate its effect on the extraction yield of polysaccharides when other parameters were set as follows: extraction time 90 min, extraction temperature 80°C. When ratio of water to raw material is 60 mL/g, the polysaccharide extraction yield is the largest. From 50 to 60 times, *Lentinula edodes* polysaccharides yield showed a trend of rapid rise. When the material liquid than from 60 to 80 times, with the yield is reduced with the increase of the amount of water (Fig. 4). This is because the water content, the more the greater the dispersion in water samples, the greater the contact area, to extract; But after add water to a certain extent, add water, dispersion is too big, is not conducive to extract, thus caused the continue to improve water and polysaccharide yield decline.

Table 3: Results of variance analysis for regression equation

Source	DF	S.S.	M.S.	F value	p-value prob>F	Significant
Model	9	33.650	3.740	25.16	0.0002	**
A	1	2.600	2.600	17.49	0.0041	**
B	1	1.620	1.620	10.90	0.0131	*
C	1	2.120	2.120	14.28	0.0069	**
AB	1	0.860	0.860	5.82	0.0466	*
AC	1	0.400	0.400	2.67	0.1462	
BC	1	0.029	0.029	0.19	0.6725	
A^2	1	4.290	4.290	28.88	0.0010	**
B^2	1	6.840	6.840	46.03	0.0003	**
C^2	1	12.300	13.300	82.81	<0.0001	**
Residual	7	1.040	0.150			
Lack of fit	3	1.040	0.350	2665.64	<0.0001	**
Pure error	4	5.200E-004	1.300E-004			

**: "Prob>F" value is less than 0.01 represents a significant; *: Remarkable, less than 0.05; S.S.: Sum of square; M.S.: Mean square

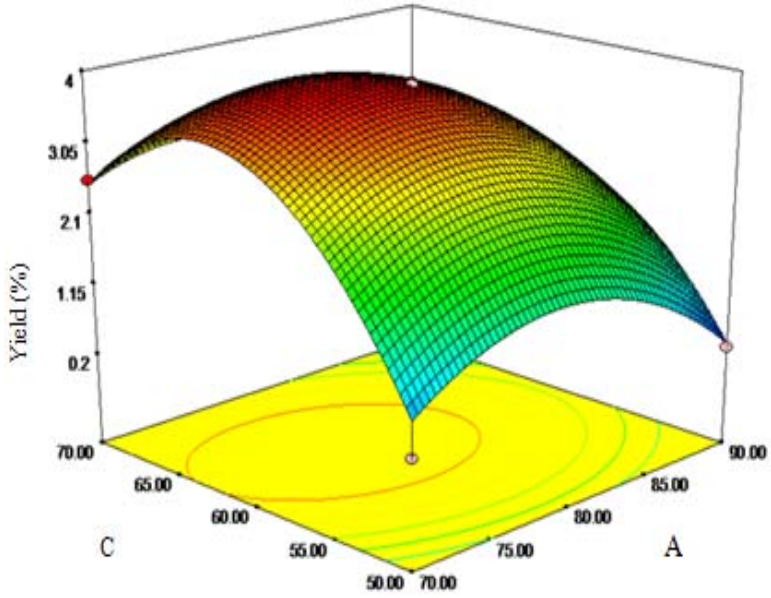

Fig. 5: Responsive surfaces and contours of Y = f (A, C)

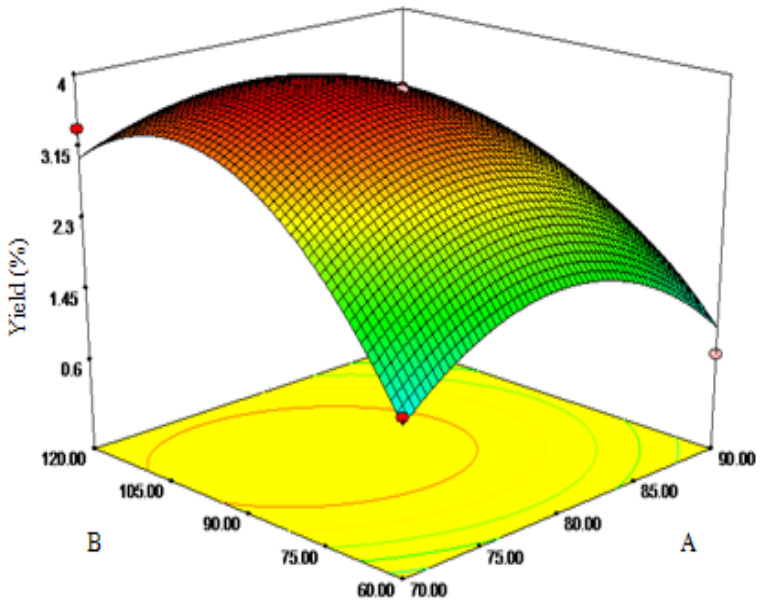

Fig. 6: Responsive surfaces and contours of Y = f (A, B)

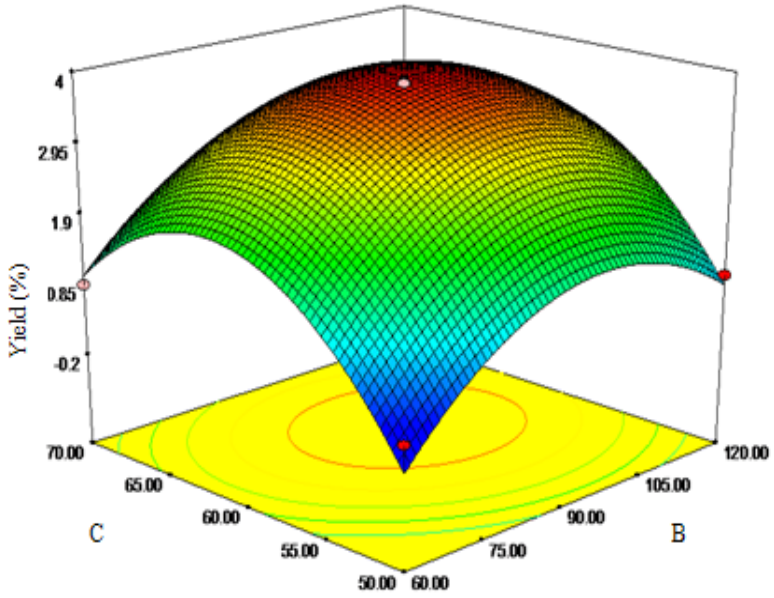

Fig. 7: Responsive surfaces and contours of Y = f (B, C)

Optimization of extraction conditions for *Lentinula edodes* polysaccharides: The extraction conditions certainly affected the extraction yield of *Lentinula edodes* polysaccharides. The experiments were designed to evaluate the impact of three factors, extraction temperature (A), extraction time (B) and ratio of water to raw material (C) on polysaccharides extraction from *Lentinula edodes*. Experiment scheme are shown in Table 2. Using the Design-Expert statistical software, to simulate multiple regressions of experimental data in Table 2, regression analysis results are shown in Table 3. *Lentinula edodes* polysaccharides extraction rate of extraction temperature and time and ratio of water to raw material than quadratic multinomial regression equation of various independent variables:

$$Y = 3.83 - 0.57A + 0.45B + 0.52C - 0.46AB - 0.32AC - 0.0085\ BC - 1.01A2 - 1.27B2 - 1.17C2$$

$$R^2 = 0.9315$$

R^2 is 0.9315, the response value (*Lentinula edodes* polysaccharides extraction yield) changes with 93.15% comes from the selected variables, namely, temperature, ratio of water to raw material, time.

Table 3 shows that using the regression equation to describe the factors and the relationship between the response value, a linear relationship between the dependent variable and the independent variables $R^2 = 0.9315$. Model equation regression significantly, equation of quasi p values, shows that this method is reliable. The equation fitting well with the result of the experiment, using the equation instead of real laboratory analysis and forecasting is feasible.

According to the analysis result, extraction temperature and ratio of water to material of primary and secondary influence on *Lentinula edodes* polysaccharides yield reached extremely significant level ($p<0.01$), an item of lentinan extracting time yield significantly ($p<0.05$) and the secondary effects of extremely significant level ($p<0.01$), extraction temperature and time of interaction of *Lentinula edodes* polysaccharides yield significantly ($p<0.05$), the single factor effect on polysaccharide yield greatly, affect the primary and secondary to A>C>B, less influence of the interaction between other factors. The response surface analysis results as shown in Fig. 5 to 7.

Figure 5 to 7 show the interaction among various factors affect response values LEP extraction yield of a multiple quadratic regression equation for partial derivatives, can get:

$$0.57 + 0.46B + 0.32C - 2.02A = 0$$

$$0.45 - 0.46A - 0.01C - 2.5B = 0$$

$$0.52 - 0.32A - 0.01B - 2.2C = 0$$

And then simultaneous equations, solution hot water leaching lentinan optimal condition code value is as follows:

$$A = 0.34,\ B = 0.12,\ C = 0.19$$

Extraction temperature (A), extraction time (B) and ratio of water to raw material (C) code value and experimental value between the conversion formula is:

$$A = (Z1-80)/10,\ B = (Z2-90)/30,\ C = (Z3-60)/10$$

Transform coding value into type:

Z1 = 76.6°C, Z2 = 88.8 min, Z3 = 58.1 (mL/g)

Regression model to predict the rate of 3.83%. Namely in extraction temperature is 76.6°C, the extraction time was 88.8 min, ratio of water to raw material of 58.1 (mL/g), models to predict the *Lentinula edodes* polysaccharides yield a maximum of 3.83%.

DISCUSSION

Through the Box-Behnken design and response surface analysis, by using the statistical software design-expert can be optimized experiment and data processing in a reliable way.

This experiment by hot water extraction, extraction of lentinan can use Design-Expert software reliable optimization experiment and data processing of the experiment, the best response optimization of polysaccharide extraction time was 88.8 min, optimum temperature is 76.6°C, the best ratio of material liquid 58.1 (mL/g), to predict the best extraction yield of 3.83%. According to references did not use ultrasonic cleaning machine auxiliary *Lentinula edodes* polysaccharides obtained at a rate of 2.51%, this experiment using the ultrasonic cleaners auxiliary experiment can improve the yield of polysaccharide, the best rate of 3.83%. Ultrasonic-assisted extraction suitable for the extraction of lentinan, compared with the conventional water *Lentinula edodes* polysaccharides extraction, extraction rate is high, the time is short, less energy consumption, easy to operate.

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