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Short communication

A comparison study on extraction of polysaccharides from *Tricholoma matsutake* by response surface methodology

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ABSTRACT

The *Tricholoma matsutake* polysaccharides (TMP) extraction was optimized using counter-current probe ultrasonic extraction (CCPUE) by response surface methodology (RSM). The effect of three factors (liquid–solid ratio, extraction time and pulse duration) on the TMP yield was studied. The results showed that the optimal conditions were liquid–solid ratio (15), extraction time (61 min) and pulse duration (6.0 s). Under best conditions, the maximum TMP yield was $8.11 \pm 0.35\%$, which was close with the predicted yield value (8.03%). The five methods, hot-water extraction (HWE), enzyme assisted extraction (EAE), ultrasonic clearer extraction (UCE), static probe ultrasonic extraction (SPUE), and CCPUE for extracting TMP by RSM were further compared. The results indicated that CCPUE had the largest yield of TMP with modest energy consumption.

1. Introduction

Tricholoma matsutake, which is an ectomycorrhizal commensal fungi, is a kind of valuable mushroom belonging to subgenus *Tricholoma*. The fruiting body of *T. Matsutake* is very important edible mushroom, which is nutrimental and delicious. The polysaccharides of *T. Matsutake* have multiple immunostimulatory activities (Byeon et al., 2009), antineoplasmic activity (Ding et al., 2010), antioxidation (Kim, Lee, Cho, Kim, & Hong, 2010), hematopoietic activities and antimutation (Yin, You, & Jiang, 2011). It has been used for the prevention and treatment of diseases for more than 1000 years in Asian countries, such as Korea, China, and Japan (Yin et al., 2011).

Hot-water extraction (HWE) has been widely used to extract mushroom polysaccharides, however, the longer extraction time and higher temperature used in HWE than other extraction methods may result in a decrease in the pharmacological activity of extracted mushroom polysaccharides (Yin, You, & Jiang, 2012; You, Yin, & Ji, 2014; You, Yin, Zhang, & Jiang, 2014; You, Yin, & Zhao, 2013).

Various recently developed novel techniques for the extraction of bioactive polysaccharides from mushrooms, include EAE, UCE and ultrasonic-assisted extraction (UAE). Among of these methods, UAE has the advantage of accelerating the speed of extraction, decreasing damage to the structural and molecular properties of polysaccharides (You et al., 2014a). In this study, five extraction methods, including HWE, UCE, EAE, static probe ultrasonic extraction (SPUE), and CCPUE, were compared by the yields of TMP and energy consumption of the five extraction methods.

2. Materials and methods

2.1. Materials

The fruiting bodies of the *T. Matsutake* were purchased from market. Samples were dried at 60 °C for 12 h and smashed by disintegrator and were sieved by 60 mesh sifter. The dried samples was firstly de-fatted with petroleum ether (60–90 °C) and pretreated with 80% ethanol to remove oligosaccharides, small-molecule materials, and some colored substances, then, the pretreated samples were separated from the miscible liquids by centrifugation ($5000 \times g$ for 10 min) and were dried.

2.2. Extraction of TMP by various methods

Extraction method of HWE and EAE was according to the reference (You et al., 2013), and extraction method of UCE, SPUE, and

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Table 1
Independent variables and their levels used in the response surface design.

Independent variables	Factor level		
	−1	0	1
Liquid–solid ratio (X_1)	10	15	20
Extraction time (X_2, min)	40	60	80
Pulse duration (X_3, s)	5	6	7

CCPUE was according to the reference (You et al., 2014a). An ultrasonic clearer with a power of 200 W and a frequency of 20 kHz was used in the UCE method. The instruments of SUPE and CCPUE were almost the same and the difference were that in the CCPUE method two peristaltic pumps were used to keep the material solutions in a counter-current flow state. The interval times for SPUE, CCPUE were 2 s.

The pretreated sample was extracted using the methods described above. After extraction, the insoluble residue was removed by vacuum filtration from the solutions, and the filter liquor was concentrated to about one quarter of the initial volume using a rotary evaporator at 50 °C under vacuum. After cooling, proteins were completely removed by the repeatedly addition of trichloroacetic acid (Wang, Jiang, Wang, & Li, 2013). The supernatant was precipitated by the addition of ethanol to a final concentration of 80% (v/v) and the precipitates were gathered by centrifugation for 10 min at 8000 rpm in a high-speed centrifuge and subsequently lyophilized.

The polysaccharides yield (%) is calculated using the following formula:

$$\text{TMP yield}\,(\%) = \frac{\text{the polysaccharides content of extraction (g)}}{\text{weight of} T. \ \textit{matsutake} \text{ powder (g)}} \times 100\% \quad (1)$$

2.3. Experimental design of CCPUE of TMP and analysis

The extraction parameters were optimized by RSM. The behavior of the TMP extraction was explained by following model (Yin et al., 2011):

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

y is the response function, β_{k0} is an intercept, 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. The fitted polynomial equation is expressed as surface and contour plots in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions (Lu, Engelmann, Lila, & Erdman, 2008).

3. Results and discussion

3.1. Optimization of the CCPUE conditions

RSM extractions of CCPUE conditions were implemented on the basis of one-factor experimental design. The range and center point values of three independent variables (Table 1) were based on the results of preliminary experiments. A total of 16 runs for optimizing the four parameters is in the BBD, the extraction conditions and the yield of TMP according to BBD was shown in Table 2.

Experiment results indicated that the yield of TMP ranged from 4.94 to 8.02%. The maximum extract yield of 8.03% was found in conditions of $X_1 = 14.69823$, $X_2 = 60.96336$ min and $X_4 = 6.31064$ s. The results were fitted with one basic quadratic equation. The date of regression coefficients were computed, the response variable and the test variables are related by the following quadratic equation:

Table 2
RSM design and TMP yield.

Run	Liquid–solid ratio (X_1)	Extraction time (X_2, min)	Pulse duration (X_4, s)	TMP yield (Y, %)
1	10	40	6	6.61
2	20	40	6	6.27
3	10	80	6	6.76
4	20	80	6	6.73
5	10	60	5	6.99
6	20	60	5	4.94
7	10	60	7	7.11
8	20	60	7	7.48
9	15	40	5	5.66
10	15	80	5	6.30
11	15	40	7	6.80
12	15	80	7	6.68
13	15	60	6	8.02
14	15	60	6	7.91
15	15	60	6	7.82
16	15	60	6	7.98

$$\begin{aligned} Y = {} & -26.3800 - 0.1818X_1 + 0.2939X_2 + 8.4875X_3 - 0.0214X_1^2 \\ & -0.0020X_2^2 - 0.7675X_3^2 + 0.0008X_1X_2 + 0.1210X_1X_3 \\ & -0.0095X_2X_3 \end{aligned} \quad (3)$$

where X_1, X_2 and X_3 are the variables for liquid–solid ratio, extraction time and pulse duration, respectively, Y represents the yield of TMP (%). The analysis of variance (ANOVA) indicated that the linear coefficients (X_1, X_2, X_3), quadratic coefficients (X_1^2, X_2^2, X_3^2) and interaction coefficient ($X_1 \times X_3$, $X_2 \times X_3$) were significant, with small p-values ($p < 0.03$). The equation regression coefficient value ($R^2 = 0.96353$) indicated that this equation was valid. Eq. (3) was made three dimensional and contour plots to predict the relationships between the independent variables and the dependent variables. The best values of the yield of TMP (8.03%) can be calculated as follows: liquid–solid ratio (14.69823); extraction time (60.96336 min) and pulse duration (6.31064 s). Among all three factors, pulse duration was the most significant variable, followed by liquid–solid ratio and extraction time according to the regression coefficients significance of the equation and gradient of slope in the 3D response surface map (Fig. 1). The usability of the model was checked by using the selected best conditions. The optimum conditions can be modified as follows: liquid–solid ratio (15), extraction time (61 min) and pulse duration (6.0 s). Under optimal conditions, the yield of TMP is 8.11 ± 0.35% ($n = 3$), which was well-matched with the predicted value (8.03%) obtained from the model. The result demonstrates the validation of the model.

3.2. Comparison of HWE, UCE, EAE, SUPE and CCPUE

RSM were similarly applied for optimizing the extraction process of HWE, UCE, EAE, SUPE. The TMP yield and energy consumption of HWE, UCE, EAE, SUPE were contrasted in Table 3. The yield of TMP by CCPUE was highest among of these methods. This indicated that CCPUE had higher extraction efficiency. The energy consumptions of the four extraction methods were analyzed using a DDS666-electronic single-phase power meter (China Instrument & Meter Co., Ltd, Zhejiang, China). Energy consumption data were read when the TMP yield was about 5.00%. The HWE method demands the highest energy consumption (0.53 kWh) because of high temperature extraction (Table 3). However, CCPUE needs only energy consumption (0.34 kWh), What's more it is extraction time is the shortest. The possible reason for this phenomenon is that the CCPUE system comprises two parts: ultrasound equipment and

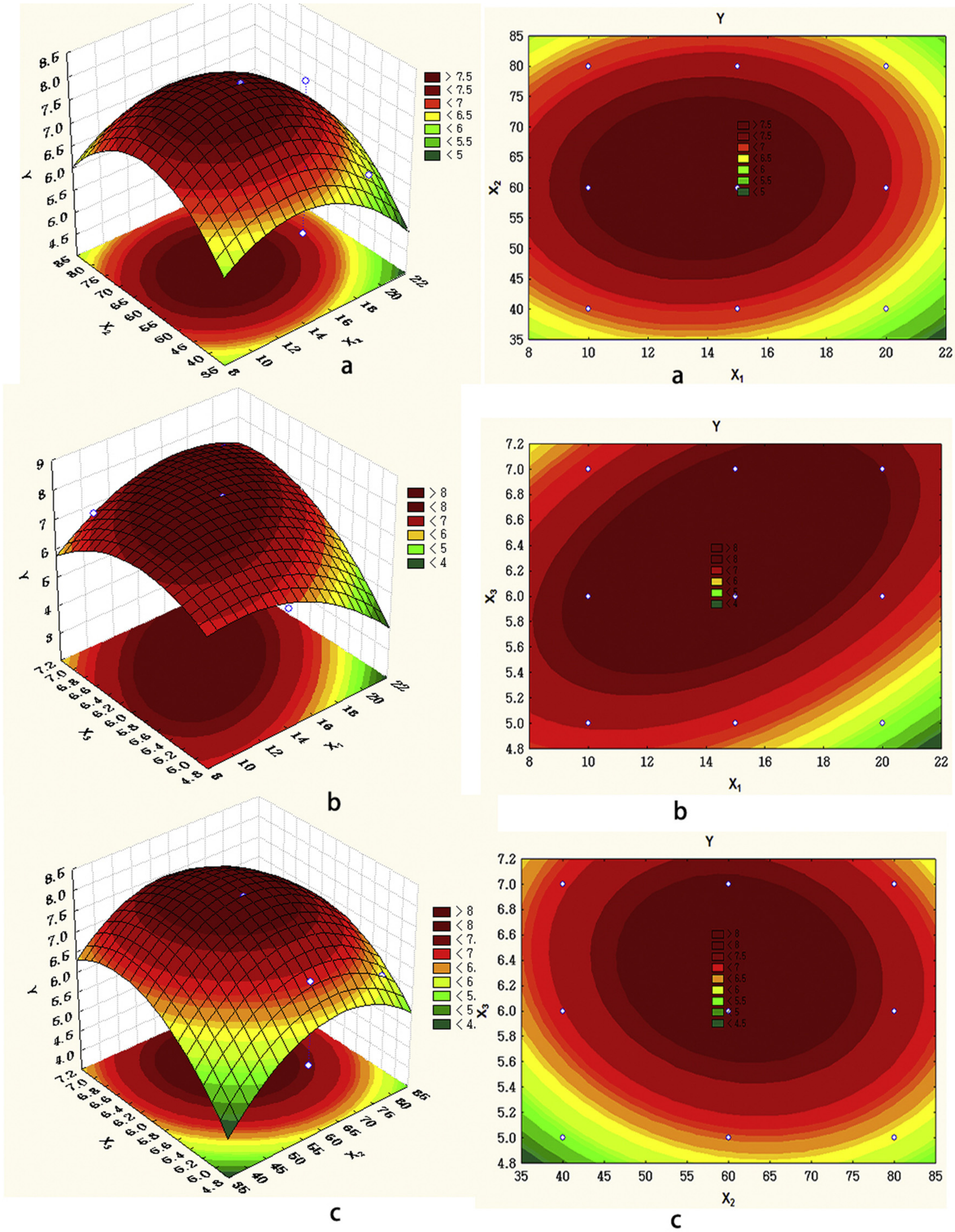

Fig. 1. Response surface (3D) and contour plots showing the effect of the liquid–solid ratio (X_1) and extraction time (X_2) on the TMP yield response (Y) (a); liquid–solid ratio (X_1) and pulse duration (X_3) on the TMP yield response (Y) (b); extraction time (X_2) and pulse duration (X_3) on the TMP yield response (Y) (c).

Table 3
Comparison of the yields and energy consumptions of five methods.

Method	HWE	UCE	EAE	SUPE	CCPUE
Equipment costs	Low	Medium	Low	High	High
Operator skill	Low	Low	Medium	Medium	Medium
Energy consumption (kWh)	0.53 ± 0.03[a]	0.38 ± 0.03	0.29 ± 0.02	0.32 ± 0.02	0.34 ± 0.02
TMP yield (%)					
Predicted (%)	5.60	6.56	7.65	7.95	8.02
Experimental (%)	5.52 ± 0.11	6.47 ± 0.23	7.53 ± 0.26	7.97 ± 0.31	8.11 ± 0.35

[a] Mean ± standard deviation (n = 3).

peristaltic pumps. The energy consumption for EAE and SPUE was less than that of CCPUE; however, the two methods need longer extraction times than CCPUE. Among of these methods, CCPUE is the best extraction method because of its relatively low energy consumption and high extraction efficiency. These results indicated that CCPUE was a very promising extraction method that offer high extraction efficiency and relatively low energy consumption.

4. Conclusion

CCPUE was employed to extract TMP polysaccharides by RSM. The results indicated that a best TMP yield could be obtained when extraction conditions were: liquid–solid ratio (15), extraction time (61 min) and pulse duration (6.0 s). Under best conditions, the maximum TMP yield was $8.11 \pm 0.35\%$, which was close with the predicted yield value. Among of UCE, EAE, SUPE and CCPUE, CCPUE was a very new promising extraction method.

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