Asian Journal of Chemistry; Vol. 25, No. 14 (2013), 0000-0000

ASIAN JOURNAL OF CHEMISTRY

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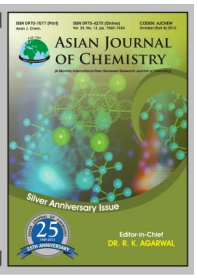


Optimization of Microwave-Assisted Extraction of Cordycepic Acid and Cordycepin from Cultured *Cordyceps militaris* by Response Surface Methodology

LI DENG[1,2,3], TONG-YONG ZHOU[1,2,3], LI PI[1,2,3], XIAO-HUI ZHAO[1,2,3], TAO HAN[1,2,3], YI-KANG LI[1,2,3] and FA HAN[1,3,*]

[1]Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, P.R. China
[2]Graduate University of Chinese Academy of Sciences, Beijing 100049, P.R. China
[3]Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, P.R. China

*Corresponding author: E-mail: designdl@163.com

(*Received*: ; *Accepted*:) AJC-0000

The response surface methodology was employed to optimize the integrated extraction parameters of cordycepic acid and cordycepin from cultured *Cordyceps militaris* (L.) link based on a single-factor experiment. The Box-Behnken design with three independent variables *i.e.*, microwave power (W), water/material ratio (mL/g) and extraction time (min) was used. The experimental data obtained were fitted to a second-order polynomial equation using multiple regression analysis. The three-dimensional response surface plot and contour plot derived from the mathematical models were applied to determine the optimal conditions. The optimum extraction condition was obtained as follows: microwave-assisted extraction, microwave power of 649.33 W, extraction time of 5.74 min, water/material ratio of 38. 99 mL/g and extraction number of three. The yield of cordycepic acid and cordycepin were 2.47 and 0.79 %, respectively. Under these conditions, the experimental values of 3.12 and 0.75 % well agreed with those predicted by the model.

Key Words: *Cordyceps militaris*, Cordycepin, Cordycepic acid, Microwave-assisted extraction, Reponse surface methodology.

INTRODUCTION

Cordyceps militaris is an edible and medicinal fungus that belongs to Clavicipitaceae family. Recent studies have indicated that *C. militaris* has both special nutritional and obvious medicinal value[1,2]. *C. militaris* has various pharmacological activities attributed to polysaccharide and cordycepin contents[3]. Cordycepin (3'- deoxyadebosine) inhibits DNA and RNA syntheses, enhances cell differentiation, as well as exhibits antitumor, antifungal and antibacterial activities[4]. Cordycepic acid has antihepatic fibrosis, antilipid peroxidation and antibacterial effects[5].

In recent years, *C. militaris* has attracted considerable attention. It has been extensively cultivated and developed in many areas. A large number of drugs and health foods have been marketed, although most of them lack market competitiveness[5]. Therefore, the active components in the products of *C. militaris* must be improved to ensure their steady and sustainable development. Currently, domestic and international enterprises and research institutes use single-component extraction methods for extracting the effective components of *C. militaris*, which limit the potential and orientation of the deeply processed products of *C. militaris*. Therefore, studies on the integrated extraction process of active components from *C. militaris* and the improvement of their yield have important economic value and cover a wide potential application market.

The microwave-assisted, ultrasonic, refluxing and Soxhlet extraction methods are currently used to extract cordycepin[6]. The microwave-assisted and ultrasonic extraction methods are used to extract cordycepic acid[7]. In recent years, the microwave-assisted extraction method has been used to extract active components from *C. militaris* because it is fast, energy saving, solvent saving and causes little pollution[8]. Some researchers have studied the improvement and optimization of integrated extraction processes for cordycepic acid and cordycepin. However, the optimum process was obtained under single-factor and orthogonal experiments[7-10]. The response surface methodology (RSM) is an effective method for optimizing process conditions. The RSM can determine the relationship between one or multiple response variables and a series of tests variables, indentifying the impact of experimental factors and their interaction on the indicator response in the process and accurately describing the relationship between the factors and response values[11]. In present study, the integrated extraction process of cordycepic acid and cordycepin in cultured *C. militaris* were optimized using the RSM. The

experimental data were analyzed by solving the regression equation with design expert software to provide reliable technical parameters and theoretical foundation for commercial processes.

EXPERIMENTAL

Cultured *C. militaris* was obtained from the Xining Shifeng Bioengineering Corporation, Xining, Qinghai Province, China. The content of cordycepic acid and cordycepin in the samples were 7.193 and 1.336 %, respectively. The material was identified at the Institute of Microbiology Chinese Academy of Sciences, Beijing, China. Cordycepin and mannitol standards were purchased from the National Institutes for Food and Drug Control. All solvents were (high-performance liquid chromatogprahy grade) and purchased from Beijing Chemical Corporation (Beijing, China). All other chemicals were analytical grade and from Yuwang Regents Corporation (Shandong, China), unless otherwise stated.

Preparation of samples: The fruiting bodies of *C. millitaris* were ground in a blender to obtain a fine powder (60-mesh size screen) after drying at 60 ºC. The powder was defatted by Soxhlet extraction with *n*-hexane as the solvent. The defatted power was placed at room temperature over-night to allow the release of residual *n*-hexane and then packaged and stored in the dark at room temperature until used[9,14]. Subsequently, 10 g of defatted powder was immersed into the extraction solution containing 300 mL distilled water and the extracted in the microwave oven (NJL07-3, China) at 50 ºC for 4 min (Fig. 1). The sample extraction procedure was repeated thrice. After cooling the filtrates, the filtrate was combined and concentrated to constant volume with a rotary evaporator at 60 ºC under vacuum. The concentrated filtrate was precipitated with acetone. The acetone supernatant was decanted and the precipitate was collected by centrifugation, dried *in vacuo*, dissolved in distilled water[9,14]. The aqueous solution was precipitated two times with acetone. This procedure was repeated thrice and the final precipitate was dissolved in distilled water. All acetone supernatants were combined together and settled to constant volume. The precipitate was used to determine the cordycepic acid content by colorimetry and the acetone supernatant was used for HPLC analysis.

Fig. 1 Microwave equipment

Determination of cordycepic acid content: The cordycepic acid content was determined using the colorimetry method. About 0.5 mL of appropriately diluted sample was mixed with 1 mL of potassium periodate solution, which was allowed to stand at room temperature for 10 min. Subsequently, 2 mL of L-rhamnose solution was added to the mixture. The freshly prepared NASH reagents (150 g ammonium acetate + 2 mL glacial acetic acid + 2 mL acetylacetone) were added to the mixture after vigorous shaking. The mixture was placed in a 35 ºC water bath for 15 min and then cooled rapidly to room temperature. The absorbance of the mixture was measured at 415 nm against a reagent blank (0.5 mL of distilled water instead of the sample) using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). A standard curve was prepared using mannitol and the linear regression equation

$$A = 0.008C + 0.0203, R^2 = 0.9995$$

linear range equal to 10 to 50 µg/mL. The percentage cordycepic acid extraction yield (%) was calculated as the cordycepic acid content of extraction divided by dried sample weight (10 g).

HPLC assay of cordycepin content: Cordycepin was determined by HPLC according to a reported procedure[13]. HPLC analysis was performed on an Agilent1200 liquid chromatography system (Agilent Technologies, USA), equipped with a vacuum degasser, four single solvent delivery pumps, a thermostatted column compartment, a 20 µL sample loop manual injector and a diode-array detector. The HPLC conditions were as follows: column, Agilent symmetry C_{18} (250 mm × 4.6 mm, 5 µm particle size); mobile phase, a mixture of methanol and water (12:88, v/v); flow rate, 0.8 mL/min; UV detection wavelength at 260 nm and injection amount, 10 µL. The samples were filtered through a 0.45 µm membrane filter before injection. The detected peak was identified by comparing the retention times with the standard. Quantitative analysis was determined using the peak area based on the standard curves. A standard curve was prepared and the linear regression equation

$$A = 35115C - 16.898, R^2 = 0.9996$$

linear range equal to 0.50×10^{-2}-3.50×10^{-2} µg/mL. The percentage cordycepin extraction yield (%) was calculated as the cordycepicn content of extraction divided by dried sample weight (10 g).

Single-factor experiment: In this study, single-factor experiment was applied to select the appropriate extraction conditions (extraction methods, extraction number, microwave power, water/material ratio and time) for the extraction of cordycepic acid and cordycepin from cultured *C. millitaris*. Ultrasonic assisted extraction and microwave-assisted extraction methods were used to determine the optimal method for extracting cordycepic acid and cordycepin from cultured *C. militaris*. The defatted powder (1 g) was immersed into the extraction solution containing 30 mL distilled water and extracted with ultrosonic treatment (100 W) for 0.5 h at 60 ºC. 10 g of defatted powder was immersed into the extraction solution containing 300 mL distilled water and extracted with microwave treatment (500 W) for 4 min. The second step of the single-factor experiment was to determine the effect of the number of extraction on the yields of cordycepic acid and

TABLE-1
EFFECT OF DIFFERENT EXTRACTION METHODS ON CORDYCEPIC ACID AND CORDYCEPIN YIELD OF *C. militaris*

Extraction method	Sample quantity (g)	Extraction time (min)	Ratio of solution to solid (mL/g)	Yield (%)	
				Cordycepic acid	Cordycepin
UAE	10.0	30	30	2.35	0.65
MAE	10.0	4	30	2.67	0.74

cordycepin. The final step was to evaluate the appropriate microwave power, water/material ratio and duration of extraction. All single-factors were repeated thrice.

Experimental design: After determining the preliminary range of extraction variables *via* single-factor experiments, the RSM was applied to identify the optimum levels of three variables *i.e.*, microwave power (W), extraction time (min) and water/material ratio (mL/g) for obtaining the best yields of cordycepic acid and cordycepin from the cultured *C. militaris* extracts. The independent variables used in the RSM design are listed in Table-2. The range and central point values of microwave power (x_1), time (x_2) and water/material ratio (x_3) were selected based on the single-factor experimental results. The experiments had a Box-Behnken design (BBD) with three central points as shown in Table-3. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

TABLE-2
UNCODED AND CODED LEVELS OF INDEPENDENT VARIABLES USED IN THE RSM DESIGN

Symbols	Independent variables	Coded levels		
		-1	0	1
x_1	Microwave power (w)	300	500	700
x_2	Extraction time (min)	2	4	6
x_3	Water/material ratio (mL/g)	20	35	50

The variables were coded according to the following equation:

$$x = \left(\frac{(X_i - X_o)}{\Delta X} \right) \quad (1)$$

where x is the coded value, X_i is the corresponding actual value, X_o is the actual value in the centre of the domain and ΔX is the increment of X_i corresponding to a variation of one unit of x. A second-order polynomial equation was used to express the responses as a function of the independent variables as follows:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x^2_1 + \beta_{22} x^2_2 + \beta_{33} x^2_3 \quad (2)$$

where Y represents the measured response variables, β_0 is a constant and β_1, β_2, β_3, β_{12}, β_{13}, β_{23}, β_{11}, β_{22} and β_{33} are the linear, quadratic and interactive coefficients of the equation, x_1, x_2 and x_3 are the levels of the independent variables. Analysis of the experimental design data and calculation of the predicted responses were carried out using Design Expert software (Version 7.0). Additional confirmation experiments were subsequently conducted to verify the validity of the statistical experimental design.

RESULTS AND DISCUSSION

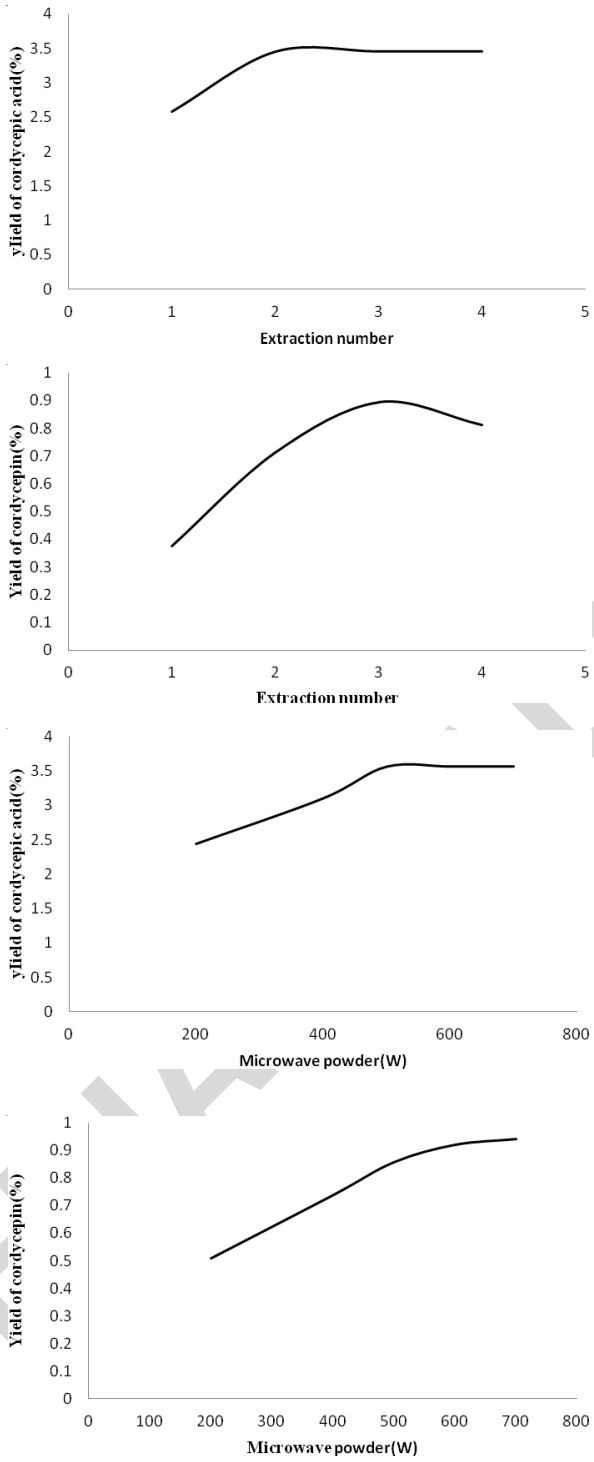
Single-factor experiment: To determine the optimal method for extracting cordycepic acid and cordycepin, ultrasonic water and microwave-assisted extraction methods were employed. As shown in Table-1, the two methods adequately extracted cordycepic acid and cordycepin. The extraction yields of cordycepic acid and cordycepin by microwave-assisted were better than that by ultrasonic water extraction. Microwave assisted extraction is a relatively new method and is receiving increasing attention as an alternative to current methods. Microwave assisted extraction can greatly reduce the extraction time for the same level of extraction, the quantity of solvent is less and the processing time is shorter. The high efficiency of microwave assisted extraction found in this work was suggested to be because the cells of *C. militaris* were broken by the microwave radiation, so that cordycepic acid and cordycepin dissolved

TABLE-3
EXPERIMENTAL DESIGN AND RESPONSES OF THE DEPENDENT VARIABLES TO THE EXTRACT PARAMETERS

Number	Micro-wave power X1 (W)	Time X2 (min)	Water/material ratio X3 (mL/g)	Yield (%)	
				Cordycepic acid (Y1)	Cordycepin (Y2)
1	500	2	50	1.27	0.45
2	500	4	35	2.95	0.71
3	500	4	35	3.25	0.70
4	700	2	35	1.89	0.58
5	500	6	50	1.60	0.53
6	300	4	20	2.19	0.09
7	300	6	35	2.09	0.17
8	700	4	50	1.63	0.74
9	500	4	35	3.21	0.69
10	500	4	35	3.05	0.70
11	700	4	20	3.47	0.48
12	300	4	50	1.32	0.18
13	300	2	35	0.92	0.21
14	500	2	20	2.57	0.35
15	700	6	35	2.40	0.76
16	500	4	35	3.13	0.70
17	500	6	20	3.59	0.34

more easily in the solvent.Therefore, microwave-assisted extraction was the optimal method for extracting cordycepic acid and cordycepin.

Fig. 2 shows the effect of the number of extraction on the yield of cordycepic acid and cordycepin with 500 W microwave power, 3 min extraction time and 20 mL/g water/material ratio. The yield of cordycepic acid reached the maximum value after two times of extraction and then became constant. The yield of cordycepin reached the maximum value after three times of extraction, but decreased with increased extraction times. Therefore, three extraction times was selected as the optimal number of extraction for microwave-assisted extraction.



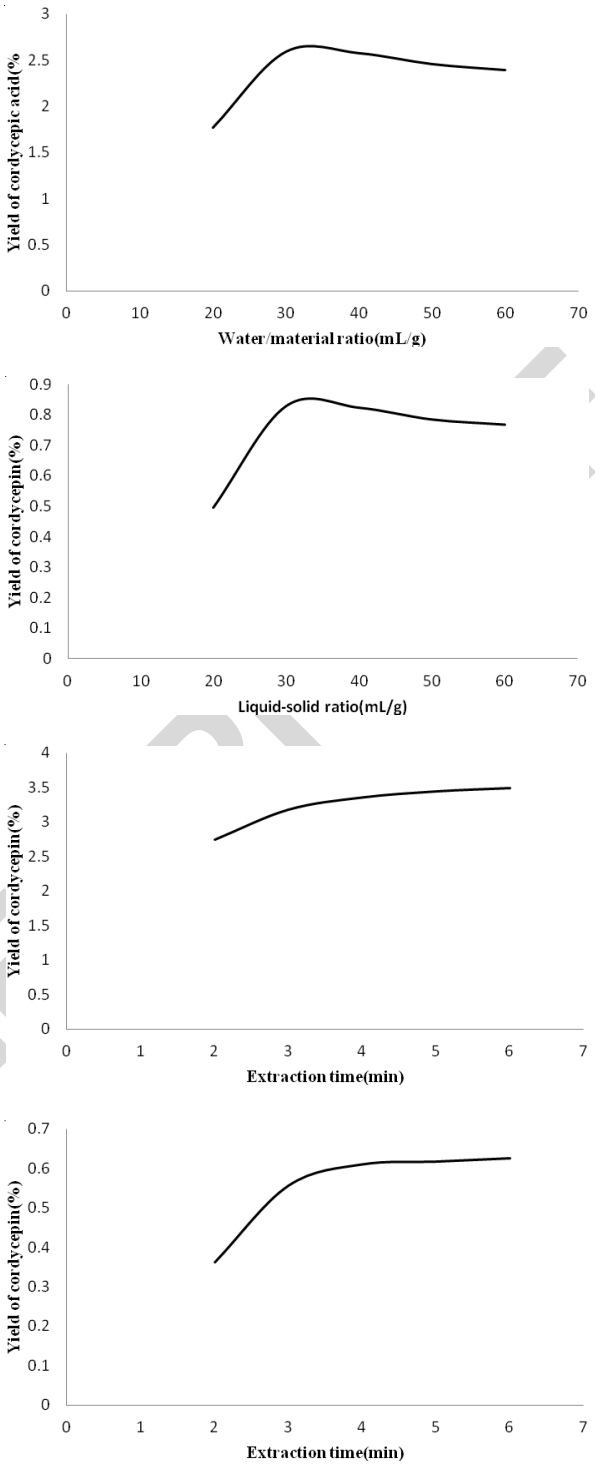


Fig. 2. Influence of extraction number, microwave power, water/material ratio and extraction time on the extraction of cordycepic acid and cordycepin from defatted powder

The effect of microwave power on the extraction yield was shown in Fig. 2. The microwave power was changed from 100-700 W and other extraction variables were set as follows: 20 mL/g water/material ratio, 3 min extraction time and three extraction times. The yield of cordycepic acid increased with increased microwave power from 200 to 500 W and then became constant. The possible reason for this result may be the complex effect of the following two aspect: firstly, the degree of disruption of the cell membrane was increased with

increasing the power and e the electric field intensity; secondly, the microwave has selective heating effect on the water-soluble polar compounds, therefore the yield of cordycepic acid and cordycepin increased with the increase of microwave power.

The yields of cordycepic acid and cordycepin extracted using different water/material ratios from 20 to 60 L/g are shown in Fig. 2. The microwave power, extraction time and extraction times were fixed at 700 W, 3 min and three extraction times, respectively. The extraction yields of cordycepic acid and cordycepin increased with the ratio until 30 mL/g and then began to decrease. The possible reason for this phenomenon may be that the loss of cordycepic acid and cordycepin were increased during the concentration process, because the dissolution of other impurities with a large quantity of water.

The extraction time is another factor that influences the extraction yield. With increased extraction time increased from 2 to 6 min, the other experimental conditions were as follows: 700 W microwave power, 30 mL/g water/material ratio and three extraction times. The extraction yield increased with increased time from 2 to 6 min, as shown in Fig. 2. A longer extraction time indicated a positive effect on the extraction yield, but the yield increased slightly. This phenomenon may be due to the active ingredients will not be dissolved when the solubility of dissolving-out substances became saturated with the increase of extraction time, while the loss of cordycepic acid and cordycepin were increased with the viscocity of extracts increased when extraction time increased. Therefore, the time range of 2 to 6 min was selected as optimal in the present experiment considering that it is cost-saving.

According to the single-parameter study, we adopted microwave-assisted extraction, 300-700 W microwave power, 20-50 mL/g water/material ratio, 2-6 min extraction time and three extraction times for the response surface methodology experiment.

Optimization of the extraction procedure by the response surface methodology

Fitting the models: The yields of cordycepic acid (Y_1) and cordycepin (Y_2) in cultured *C. militaris* extracts obtained from all the experiments are listed in Table-3. Table-4 shows the results of fitting quadratic models to the data. ANOVA indicated that the contribution of the quadratic model was significant. The fitted quadratic models for cordycepic acid and cordycepin in coded variables are given in eqns. 3 and 4, respectively. The significance of each coefficient was determined using the F-test and *p*-value (Tables 4 and 5). For all terms in the model, a large regression F-value and a small *p*-value indicate a more significant effect on the respective response variables. A lack of fit is also given in Table-4 to check the quality of the fitted models. In Table-5 the linear coefficients (X_1, X_2, X_3), a quadratic term coefficient (X^2_1, X^2_2, X^2_3) and the interaction coefficient (X_1X_3) were found significant ($p < 0.01$). There was no significance in the lack of fit ($p > 0.05$) in each of the two models, indicating that the models can be used to predict the responses.

Response surface analysis (RSA) of cordycepic acid: The response surface analysis data are given in Table-3, which that the relationship between the cordycepic acid yield and extraction parameters was quadratic with a good regression coefficient ($R^2 = 0.9980$). The value of the determination coefficient Adi-R (0.9955) suggests that only 0.45 % of the total variations are not explained by the model. Eqn. 3 shows the relationship between the cordycepic acid yield and extraction parameters.

$$Y = 3.12 + 0.36x_1 + 0.38x_2 - 0.75x_3 - 0.17x_1x_2 - 0.24x_1x_3 - 0.17x_2x_3 - 0.70x_1^2 - 0.60\,x_2^2 - 0.27x_3^2 \quad (3)$$

The effects of microwave power, extraction time and water/material ratio, on the yield of cordycepic acid, as well

TABLE-4
ANANLYSIS OF VARIANCE FOR THE RESPONSE SURFACE QUADRATIC MODEL FOR THE CORDYCEPIC ACID AND CORDYCEPIN YIELD OF *C. militaris*

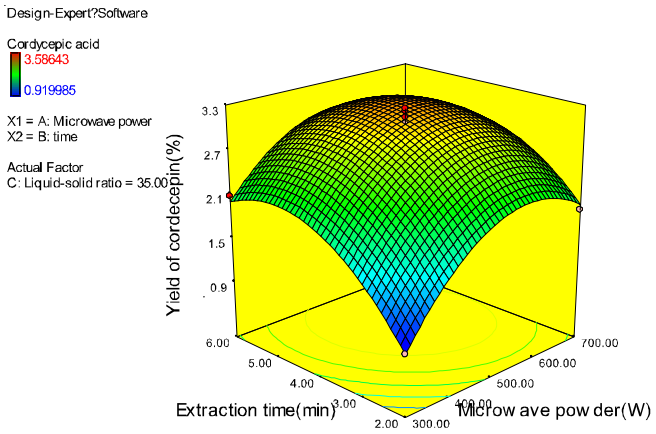
Source	DF	Cordycepic acid			Cordycepin		
		SS	F-value	*p*-Value	SS	F-Value	*p*-Value
Model	9	11.36	70.63	< 0.0001	0.84	395.98	< 0.0001
Residual	7	0.13			0.002		
Lack of fit	3	0.065	1.44	0.3551	0.001	5.27	0.0710
Pure error	4	0.06			0.0003		
Cor total	16	11.49			0.84		
–	–	$R^2 = 0.9980$ Adj-$R^2 = 0.9955$			$R^2 = 0.9891$ Adj-$R^2 = 0.9751$		

DF: Degree of freedom; SS: sum of squares.

TABLE-5
TEST OF SIGNIFICANCE FOR REGRESSION COEFFICIENT

Model term	DF	Cordycepic acid yield					Cordycepin yield				
		Coefficient estimate	Standard error	95 % Cl low	95 % Cl high	Prob > F	Coefficient estimate	Standard error	95 % Cl low	95 % Cl high	Prob > F
Intercept	1	3.12	0.06	2.98	3.26		0.7	0.007	0.68	0.72	–
X_1	1	0.36	0.047	0.25	0.47	0.0001	0.24	0.005	0.23	0.25	<0.0001
X_2	1	0.38	0.047	0.27	0.49	<0.0001	0.025	0.005	0.012	0.038	0.0024
X_3	1	-0.75	0.047	-0.86	-0.64	<0.0001	0.079	0.005	0.067	0.09	<0.0001
X_1X_2	1	-0.17	0.067	-0.32	-0.007	0.0429	0.055	0.008	0.037	0.073	0.0002
X_1X_3	1	-0.24	0.067	-0.4	-0.082	0.0089	0.04	0.008	0.022	0.058	0.0013
X_2X_3	1	-0.17	0.067	-0.33	-0.014	0.0368	0.023	0.008	0.005	0.041	0.0205
X_1^2	1	-0.7	0.065	-0.85	-0.54	<0.0001	-0.16	0.007	-0.17	-0.14	<0.0001
X_2^2	1	-0.6	0.065	-0.75	-0.44	<0.0001	-0.11	0.007	-0.13	-0.1	<0.0001
X_3^2	1	-0.27	0.065	-0.42	-0.12	0.0044	-0.17	0.007	-0.19	-0.15	<0.0001

as their interactions, are shown in Fig. 3a-c. The results reveal that the microwave power and water/material ratio had a significant positive linear effect on the yield of cordycepic acid ($p < 0.0001$). The extraction time also clearly affected the yield of cordycepic acid ($p < 0.01$). The effect of different microwave power on the extraction yield of cordycepic acid is shown in Fig. 3a-b. The extraction yield of cordycepic acid continued to increase with the increase of microwave power from 300 to 600 W and reached the peak value at 600 W. However, the extraction yield of cordycepic acid no longer increased when the microwave power exceeded 600 W. The extraction yield of cordycepic acid affected by different extraction time is shown in Fig. 3a-b. It showed that the extraction yield increased as the extraction time ascended from 3 to 5 min, the maximum yield of cordycepic acid was observed when the extraction time was 5 min, after this point, the extraction yield of cordycepic acid started to maintain a dynamic equilibrium with the increasing of the extraction time. As shown in Fig. 3b-c, when the water/material ratio was over 35 mL/g, the yield of cordycepic acid decreased gradually with increased the ratio.



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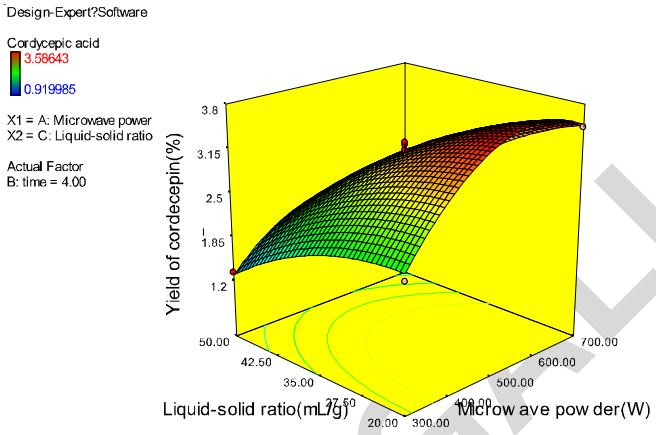

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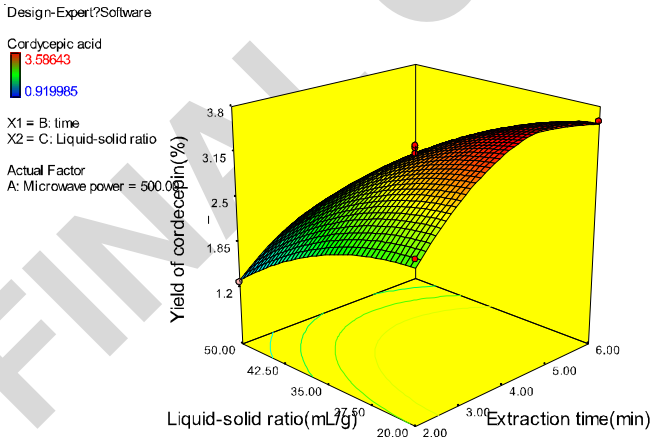


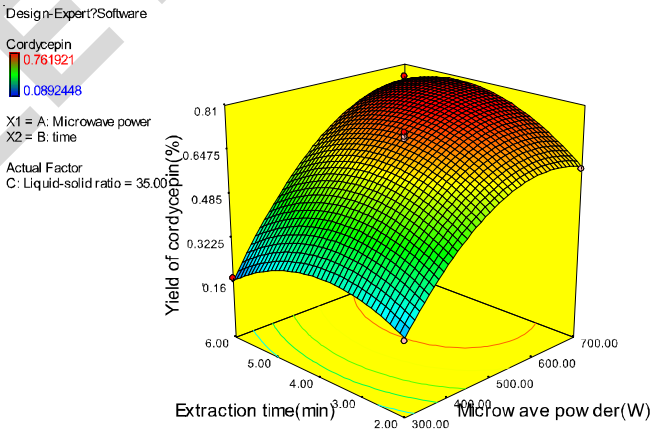
(c)

Fig. 3. Response surface plots (3-D) showing the effects of variables (X_1: microwave power; X_2: water/material ratio; X_3: extraction time) on the response Y_1

Response surface analysis of cordycepin: The response surface analysis data are given in Table-4, which demonstrated that the relationship between the cordycepin yield and extraction parameters is quadratic with a good regression coefficient ($R^2 = 0.9891$). Eqn. 4 shows the relationship between the cordycepin yield and extraction parameters.

$$Y = 0.70 + 0.24x_1 + 0.025x_2 + 0.079x_3 + 0.055x_1x_2 + 0.040x_1x_3 + 0.023x_2x_3 - 0.16x_1^2 - 0.11x_2^2 - 0.17x_3^2 \quad (4)$$

The 3-D response surface plot in Fig. 4a, give the extraction yield of cordycepin as a function of extraction time and microwave power, indicated that the extraction yield of cordycepin increased with the increasing of the microwave power from 300 to 600 W, but beyond 600 W, the extraction yield of cordycepin started to maintain a dynamic equilibrium with the increasing of the extraction microwave power and the extraction yield of cordycepin was found to increase rapidly with the increase of extraction time from 2 to 5 min, then decreased rapidly from 5 to 6 min[20].



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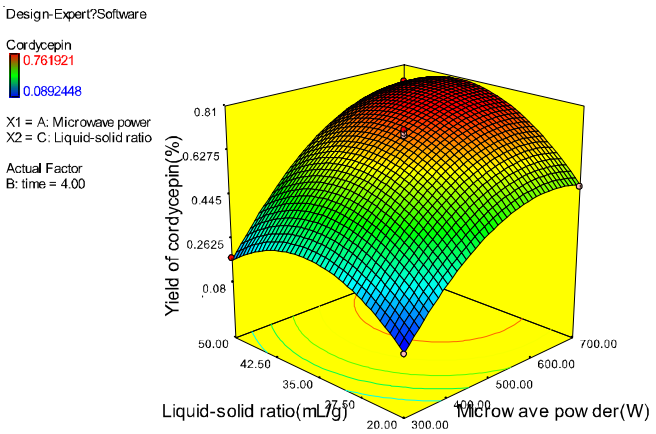

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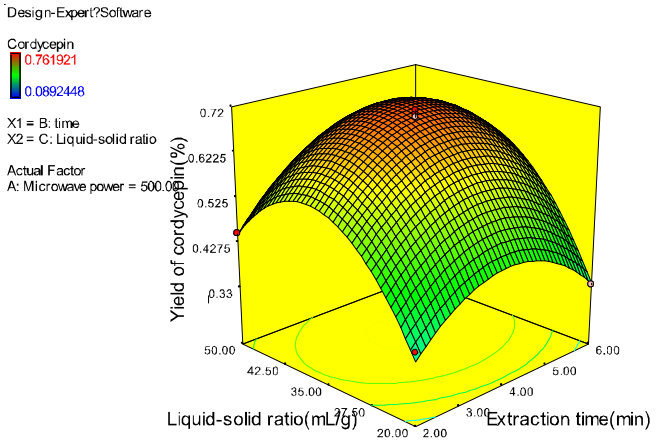

(c)

Fig. 4. Response surface plots (3-D) showing the effects of variables (X_1: microwave power; X_2: water/material ratio; X_3: extraction time) on the response Y_2

Fig. 4b showed the three-dimensional (3-D) response surface, which reveal the effect of the water/material ratio and microwave power on the cordycepin yield at the fixed extraction time of 4 min. The microwave power and water/material ratio both induced a positive quadratic effect on the yield ($p < 0.0001$). And the extraction yield of cordycepin increased rapidly within the microwave power from 300-600 W, but when beyond 600 W, the extraction yield of cordycepin reached the plateau region where the yield was maximized and did not increase any more and the yield increased rapidly with the increase of the water/material ratio from 20 to 42.5 mL/g, then dropped slightly from 42.5 to 50 mL/g.

Fig. 4c showed the 3-D response surface plots with varied extraction time and water/material ratios but fixed microwave power (zero level). The yield of cordycepin increased with increased water/material ratio and reached the maximum value when the extraction time and water/material ratio were 5 min and 40 mL/g, respectively. Beyond 5 min and 40 mL/g, the yield of cordycepin decreased.

Optimization of extraction parameters: Based on the single-factor experiments, a Box-Behnken design from the response surface methodology was used to optimize the extraction conditions in this work. The extraction conditions were deemed optimum when the yields of cordycepic acid and cordycepin reached the maximum values. Optimization was carried out using Design Expert software (Version 7.0). The values of responses were converted to a desirability function. Most effective extraction parameters for the yields of cordycepic acid and cordycepin at the same time were generated by optimizing the desirability function of the two responses. The optimum zone, in which every point represented a combination of extraction parameters that gave the optimum yields for the three dependent variables, was generated. According to practical (cost-saving) considerations, the point representing a possible combination of the lowest levels of factors within the optimum zone was preferred over other combinations. From Figs. 3 and 4, it can be concluded that the optimal extraction conditions for cordycepic acid and cordycepin from *C. militaris* are microwave power of 649.33 W, extraction time of 5.74 min, water/material ratio of 38.99 mL/g. Among the there extraction parameters that have been studied, microwave power was the most significant factor that affects the yield of cordycepic acid and cordycepin, followed by the water/material ratio and extraction time according to the regression coefficients significance of the quadratic polynomial model (Table-5) and gradient of slope in the 3-D response surface plot (Figs. 3 and 4).

Therefore, the point at the microwave power of 649.33 W, extraction time of 5.74 min, water/material ratio of 38.99 mL/g and three extraction times was considered as the optimum condition. Under this condition, the yields of cordycepic acid and cordycepin were predicted by the RSM models to be 2.47 and 0.79 %, respectively.

Verification of predicted extraction parameters: To validate the adequacy of the model equation, five verification experiments were carried out to test the suitability of the optimal extracting variables under the optimal conditions. This set of conditions was determined as optimum by the RSM optimization approach and were also used to validate experimentally as well as predict the values of the responses using the model equation. The mean values of 3.12 and 0.75 % (n = 5) obtained from real experiments indicated the validation of the RSM model. The experimental values suggested that the regression model was accurate and adequate for the extraction of cordycepic acid and cordycepin.

Conclusion

The extraction conditions have significant effects on the yields of cordycepic acid and cordycepin. Using contour and surface plots in the RSM was effective for estimating the effect of three independent variables (microwave power, water/material ratio and extraction time). The optimum set of independent variables was obtained graphically to determine the desired levels of polysaccharide and cordycepin extraction. The optimal experimental yields of 2.47 and 0.79 % were obtained when the optimum conditions of cordycepic acid and cordycepin integrated extraction were as follows: microwave-assisted extraction, 649.33 W microwave power, 5.74 min extraction time, 38.99 mL/g water/material ratio and three extraction times. Under these optimized conditions, the experimental yields of cordycepic acid and cordycepin closely agreed with the predicted yields. The experimental conditions allow a fast and cost-saving process in extraction of cordycepic acid and cordycepin from mycelia.

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