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*Research Article*

# Optimization of Vacuum-Microwave Radiation Pretreatment on Extraction of *Ganoderma* Polysaccharides

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A new process of vacuum-microwave (VM) radiation pretreatment for extracting polysaccharides from the *Ganoderma lucidum* was proposed, and the parameters were optimized by response surface methodology (RSM). The orthogonal-central composite design scheme was used and the responsive surfaces methodology of three factors and five levels was adopted, and the factors influencing the technological parameters and its interaction terms were analyzed and regressed. The optimal parameters were obtained as follows: the infiltration time of 70 min, microwave power density of 11.2 W/g, and VM irradiation time of 180 s. In consequence, the extraction yield was up to 1.775% when VM radiation was conducted in advance. Compared to the traditional hot-water extraction method, VM pretreatment can shorten the extraction time by more than a half, and the polysaccharide extraction yield was increased by 48.1%. It holds significant potential for further investigation, development, and application.

## 1. Introduction

*Ganoderma lucidum*, known for its reputation of "curiosa of immortality," is a traditional Chinese medicine with extremely high medical and nutritional value. Ancient and contemporary pharmacology and clinical research prove that *Ganoderma lucidum* does have efficacy of curing diseases and prolonging life, and modern pharmacology further confirms that the *Ganoderma* polysaccharides are the main ingredient with the effects of nourishing, strengthening, and prolonging life [1, 2].

In recent years, microwave-assisted extraction has obtained increasing attention as a potential alternative to solid-liquid extraction, and it is widely accepted in analytical laboratories as green extraction [3, 4]. Microwave-assisted extraction is conducted mainly in two methods: the first one is using the microwave extraction pot or continuous microwave extraction pipeline in which microwave is directly imported [5–8]. Usually, water and ethanol are employed as extraction solvents [9, 10]. As they have strong polarity, most of the microwave energy is consumed in rotation and vibration, resulting in low microwave efficiency. The second method is using microwave radiation pretreatment before solvent extraction, which was performed in normal pressure to extract oil from oil-bearing products [11]. However, for extraction of effective components, such as extraction of polysaccharides, flavones, and pectin, it usually leads to degradation of heat-sensitive compounds due to high temperature generated by microwave. Ethanol had ever been chosen as the vaporization agent to lower the temperature during microwave radiation pretreatment [12]. Unfortunately, ethanol vaporization has the danger of the explosion in microwave radiation.

The VM pretreatment is proposed as a novel method which involves the steps of applying reduced pressure in the enclosure during the microwave radiation to avoid the excessive temperature rise that could degrade the heat-sensitive bioactive substance [13]. In addition, water was chosen as vaporizing agent instead of ethanol, and water's temperature was controlled by vacuum pressure in the microwave cavity, to destruct plants' cell walls in low temperatures.

The objective of this study was to investigate and evaluate the new process of vacuum-microwave (VM) radiation pretreatment for extracting polysaccharides from

the *Ganoderma lucidum*. The various parameters of the VM radiation pretreatment affecting the efficiency of extraction of polysaccharides were studied using response surface methodology (RSM). An orthogonal-central composite design was used to optimise infiltration time, microwave power density, and microwave irradiation time and to evaluate the robustness of the method by drawing response surfaces. The extraction yield was also examined and compared with the traditional hot-water extraction alone.

## 2. Materials and Methods

*2.1. Samples and Regents.* The *Ganoderma* was bought from Wuxi Taihu zoology gardens, cut into pieces, and dried at 60°C to a moisture content of less than 6% in a cabinet laboratory dryer (Model: DHG-9076A, Shanghai Lab Instrument Company, Shanghai, China).

The dried *Ganoderma* pieces were further ground in a laboratory grinder (Model JIN-999, Rijin Electrical Appliance Co. Ltd., South Korea) and sieved through a 10-mesh screen (0.250 mm) to obtain a relatively homogenous sample powder.

Phenol, concentrated sulphuric acid, anhydrous alcohol, and glucose were purchased from Shanghai Chemical and Reagents Co. (Shanghai, China). All the reagents were at analytical grade.

*2.2. Experimental Rig.* The lab scale VM radiation rig in which the materials were treated is rotatable in the cavity. It was developed by the authors, and the details were described elsewhere [14–16]. The rig and microwave system had been greatly improved in this study; namely, the power output of magnetron can be continuously adjusted in the range of 0~1000 W. The vacuum pressure was also adjustable, and the rotation speed of the turntable was five rpm.

*2.3. Method of Extraction*

*2.3.1. Trial Process*

(1) A 5 g amount of sample was weighed accurately by an electronic balance (MP2000D, precision 0.01 g, Shanghai the Second Electronic Balance Instrument Co., Shanghai, China) and transferred into 250 mL conical flask. 15 mL distilled water (solid/liquid ratio 1 : 3, w/v) was added. The mixtures were soaking for 1 h at room temperature.

(2) Infiltrated sample was taken into VM rig for rapid heating pretreatment, making water infiltrated into the organization of *Ganoderma lucidum* vaporize. The cell organization is almost fully disrupted to provide a foundation for the subsequent extraction.

(3) Pretreated samples were conducted to extract, and distilled water was added as the extraction reagent according to the solid/liquid ratio of 1 : 15. Polysaccharides of *Ganoderma lucidum* were extracted in 80°C constant temperature water bath (Model DR-8D, Shanghai Jing Hong Test Equipment Co., Shanghai, China) for one hour, then filtrated, and alcohol-sunk. Finally, polysaccharide sample was obtained for later analysis.

In the above extraction process, the factors influencing extraction yield of polysaccharides are infiltration time, microwave power density, VM radiation time, vacuum pressure during the pretreatment process of microwave, and the ratio of material to water. Temperature of water and extraction time are main factors during the process of water bath extraction. The vacuum pressure controls evaporation temperature of water into the wall of cells when the microwave is radiating materials. In the test, evaporation temperature of samples heated by microwave is elected for 65°C, corresponding vacuum for −0.07 MPa. The current study of the test mainly aims at the microwave pretreatment process. The next process, namely, the process of hot-water bath extraction, can be consulted from [17, 18].

*2.3.2. Method of Traditional Hot-Water Extraction.* 5 g amount of the raw material, no pretreatment of microwave radiation, was taken and 15 g distilled water was added, soaked for one hour, and then water was joined according to the solid/liquid ratio of 1 : 15. Infiltration time and all the joined water are exactly the same as the method described in Section 2.3.1(1). Extraction temperature and time are 80°C and 2.5 h, respectively. Sample was filtrated and then alcohol-sunk. Finally, polysaccharide was obtained for testing.

*2.3.3. The Method of Analysis*

*(1) Calibration Curves*. The method for determining total water-soluble polysaccharides is based on the colored reaction of polysaccharides and its derivatives with reagents of phenol and concentrated sulphuric acid. The maximum absorption of the system is 490 nm.

D-glucan was heated at 105°C for 1 h in a cabinet dryer and then cooled down in a desiccator for 30 min. 100 mg D-glucan was weighed into 1000 mL volumetric flask, and the sample was diluted to the volume of 1000 mL with distilled water. The 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mL of the solution were piped into seven individual tubes of 10 mL with plug, respectively, and diluted to the volume of 2 mL with water. Then 1.0 mL of 6% phenol was added to each tube and shaken for two minutes. Following the above, 5.0 mL of concentrated sulphuric acid was added to each tube and shaken again for 5 minutes, respectively. In the meantime, the regent blank was prepared. The absorbance of the solutions was quantitatively measured with a colorimeter (Model 722, Shanghai Analytical Instrument Co. Ltd., Shanghai, China) at 490 nm by setting the zero absorbance with regent black.

Regression gives the linear relationship:

$$C = 0.0608A + 0.0009 \quad \left(R^2 = 0.9994\right), \tag{1}$$

Table 1: The variable factors with the coded and actual values.

| Factor | Level | | | | |
|---|---|---|---|---|---|
| | −1.682 | −1 | 0 | 1 | 1.682 |
| Infiltration time/min | 43 | 50 | 60 | 70 | 77 |
| Microwave power density/W·g$^{-1}$ | 8.5 | 9.6 | 11.2 | 12.8 | 13.9 |
| VM radiation time/s | 130 | 150 | 180 | 210 | 230 |

where $C$ (mg/mL) is the concentration of polysaccharide's solution for colorimetric analysis and $A$ is the absorbance at 490 nm.

*(2) Determination of Polysaccharides.* According to (1), the yield $Y$ was calculated by

$$Y = \frac{C \times n \times V}{m \times 10^3} \times 100\% \quad (\%), \tag{2}$$

where $V$ is the total volume of extraction solvent (mL), $m$ is the mass of sample (g), and $n$ is the multiple of dilution.

*2.4. Experimental Design.* RSM was used to optimize the VM radiation pretreatment on extraction of polysaccharides from *Ganoderma lucidum*. Experimental design and statistic analysis were conducted using SAS software. The variable factors with the coded and actual values are presented. An orthogonal-central composite design was chosen to evaluate the combined effect of three independent variables: infiltration time, microwave power density, and VM radiation time, coded as $X_1$, $X_2$, and $X_3$, respectively. The maximum and minimum values for infiltration time were set at 50 min and 70 min and microwave power density at 9.6 W/g and 12.8 W·g$^{-1}$, while 150 min and 210 min for VM radiation time (Table 1). The responses measured were extraction yields of polysaccharides from *Ganoderma lucidum*. The complete design consisted of 23 combinations, including nine replicates of the center point. Nine replicate runs at the centre of the design were performed to allow the estimation of pure error (Table 2). All experiments were carried out in a randomized order to minimize the effect of unexplained variability in the observed responses due to extraneous factors.

The responses function ($Y$) was partitioned into linear, quadratic, and interactive components [18]:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j, \tag{3}$$

where $\beta_0$ is defined as the constant, $\beta_i$ the linear coefficient, $\beta_{ii}$ the quadratic coefficient, and $\beta_{ij}$ the interactive effects' coefficient. $X_i$ and $X_j$ are levels of the independent variables while $k$ equals the number of the tested factors ($k = 3$). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic, and interaction terms were determined. The significances of all terms in the polynomial were judged statistically by computing the $F$-value at a probability ($P$) of 0.01 or 0.05. The regression coefficients were then used to make statistical calculations to generate contour maps from the regression models.

Table 2: Test design and results of RSM.

| Number | Infiltration time $X_1$ | Microwave power density $X_2$ | VM radiation time $X_3$ | Extraction yield $Y$ (%) |
|---|---|---|---|---|
| 1 | −1 | −1 | −1 | 1.661 |
| 2 | −1 | −1 | 1 | 1.589 |
| 3 | −1 | 1 | −1 | 1.665 |
| 4 | −1 | 1 | 1 | 1.689 |
| 5 | 1 | −1 | −1 | 1.725 |
| 6 | 1 | −1 | 1 | 1.628 |
| 7 | 1 | 1 | −1 | 1.707 |
| 8 | 1 | 1 | 1 | 1.681 |
| 9 | −1.68179 | 0 | 0 | 1.593 |
| 10 | 1.68179 | 0 | 0 | 1.789 |
| 11 | 0 | −1.68179 | 0 | 1.651 |
| 12 | 0 | 1.68179 | 0 | 1.646 |
| 13 | 0 | 0 | −1.68179 | 1.697 |
| 14 | 0 | 0 | 1.68179 | 1.643 |
| 15 | 0 | 0 | 0 | 1.772 |
| 16 | 0 | 0 | 0 | 1.768 |
| 17 | 0 | 0 | 0 | 1.771 |
| 18 | 0 | 0 | 0 | 1.769 |
| 19 | 0 | 0 | 0 | 1.769 |
| 20 | 0 | 0 | 0 | 1.77 |
| 21 | 0 | 0 | 0 | 1.77 |
| 22 | 0 | 0 | 0 | 1.771 |
| 23 | 0 | 0 | 0 | 1.771 |

## 3. Results and Discussion

*3.1. Model Fitting.* $P$ value is less than 0.05, which shows the item is significant. $P$ value is less than 0.01, which showed the item is extremely significant. From the ANOVA of the regression equation (Table 3), linear item and quadratic item in the equations are highly significant ($P < 0.01$). The influence of the interactive term is also significant ($P < 0.05$), so the factor and response index is not a simple linear relationship.

$R^2$ is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit. It is also the proportion of the variability in the response variables, which is accounted for by the regression analysis. The more the $R^2$ approaches unity, the better the empirical model fits the actual data [19]. Here, the $R^2$ value is 0.9151, and the regression model was highly significant ($P = 0.0001$) (Table 4), which shows that this model fits test well.

Table 3: ANOVA for the regression model.

| Source | SS | DF | MS | $F$ value | $P$ value |
|---|---|---|---|---|---|
| Linear item | 0.0221 | 3 | 0.0221 | 41.0457 | $P < 0.01$ |
| Square item | 0.0586 | 3 | 0.0586 | 108.4416 | $P < 0.01$ |
| Interactive term | 0.0048 | 3 | 0.0048 | 8.841 | $P < 0.05$ |
| Regression | 0.0849 | 9 | 0.0094 | 17.43531 | $P < 0.01$ |
| Error | 0.0070 | 13 | 0.0005 | | |
| Sum | 0.0920 | 22 | | | |

SS = sum of squares, DF = degrees of freedom, and MS = mean square.

Table 4: Result of regression analysis for RSM.

| Source | SS | DF | MS | $F$ value | $P$ value |
|---|---|---|---|---|---|
| Model | 0.0849 | 9 | 0.0094 | 17.43531 | 0.0001 |
| $X_1$ | 0.0159 | 1 | 0.0159 | 29.4629 | 0.0001 |
| $X_2$ | 0.0012 | 1 | 0.0012 | 2.3076 | 0.1527 |
| $X_3$ | 0.0050 | 1 | 0.0050 | 9.2752 | 0.0094 |
| $X_1^2$ | 0.0116 | 1 | 0.0116 | 21.5047 | 0.0005 |
| $X_1X_2$ | 0.0281 | 1 | 0.0281 | 52.0141 | 0.0001 |
| $X_1X_3$ | 0.0189 | 1 | 0.0189 | 34.9228 | 0.0001 |
| $X_2^2$ | 0.0006 | 1 | 0.0006 | 1.0997 | 0.3134 |
| $X_2X_3$ | 0.0007 | 1 | 0.0007 | 1.2993 | 0.2749 |
| $X_3^2$ | 0.0035 | 1 | 0.0035 | 6.4420 | 0.0247 |

SS = sum of squares, DF = degrees of freedom, and MS = mean square.
Explanation: $R^2 = 0.9498$; adjusted $R^2 = 0.9151$.

It means that the regression equations can replace the actual experimental data for analysis of the result.

The $P$ value of $X_1X_2$ and $X_1X_3$ is larger than 0.05 (Table 4), which means that these two interactive terms are not significant for extraction of polysaccharides. $X_1X_2$ and $X_1X_3$ were then removed. The regression model relating to the effects of infiltration time, microwave power density, and VM radiation time on extraction yield of polysaccharides from *Ganoderma lucidum* was correspondingly obtained as follows:

$$Y_1 = 1.77002 + 0.03417X_1 - 0.01917X_3 - 0.02706X_1^2 - 0.04209X_2^2 - 0.03439X_3^2 + 0.02087X_2X_3 \quad (4)$$
$$\left(\text{Adjusted } R^2 = 0.9151\right).$$

*3.2. The Effects of Factors and Its Interaction.* From Figures 1–3, it can be observed that the interactive effects of infiltration time ($X_1$), microwave power density ($X_2$), and VM radiation time ($X_3$) on extraction yield of polysaccharides were all significant. With the increase of microwave power density and VM radiation time, extraction yield of polysaccharides from *Ganoderma lucidum* improved. However, excessively high microwave power density and long microwave radiation could result in low extraction yield. This is mainly due to the high heats generated by microwave. On the one hand,

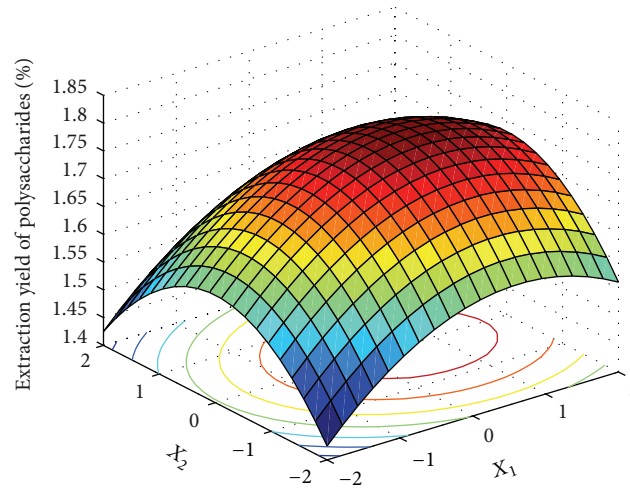


Figure 1: The effect of infiltration time and microwave power density on extraction yield of polysaccharides.

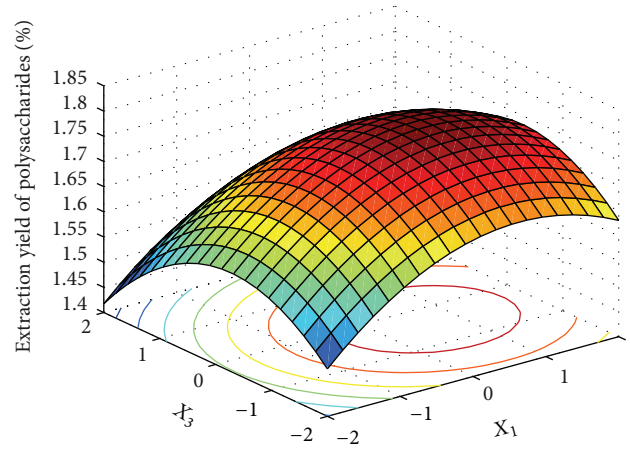


Figure 2: The effect of infiltration time and microwave radiation time on extraction yield of polysaccharides.

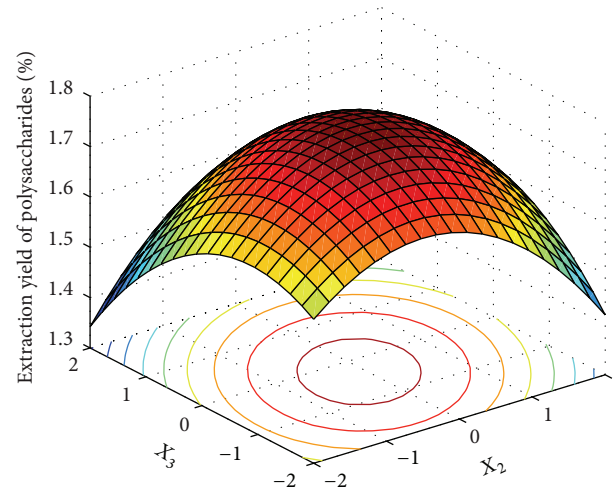


Figure 3: The effect of microwave power density and microwave radiation time on extraction yield of polysaccharides.

when using VM radiation as an assistance to extract effective component of plant, temperature-raising in biomaterial is not high, less than 65 degrees. With this method, biomass was not easily coked, and active substance's inside cell would not be destroyed. Furthermore, from the perspective of broken cells, microwave heating led polarity molecules, especially water inside the cells, to vibrate and produce a lot of heat after absorbing microwave's energy, which raised rapidly cellular internal pressure and temperature and resulted in liquid water vaporizing. The generated pressure for vaporizing made cellular membranes and walls burst sharply and formed tiny holes. By sustained rapid heating, the cell surface expanded and crack occurred. The existence of holes and crack of cell membranes rendered extracellular solvent into the cells easily and dissolved and released intracellular substances. However, the extraction yield decreased when microwave power density and VM radiation time were over about 11 W/g and 180 s, respectively. This was because the excessive high microwave power density and long VM radiation time damaged active polysaccharides due to overheating. Impurities and sticky substance dissolving made extracting solution sticky, which also obstructed the extraction of the polysaccharides.

When the infiltrating time was less than 60 min, extraction yield for polysaccharides improved along with the increase of it, which was mainly because the increase of infiltration time would ensure the definite water moving into the internal to cells resulting easier breaking the cells, and polysaccharide substances inside cells to spread outside. However, it was observed that the extraction yield changed a little if the infiltrating time was over 60 min. It could be explained that 60 min was enough for water moving into the internal cells.

Combined with the mathematical analysis of the regression model, the optimal technology parameters for VM radiation pretreatment on extraction of polysaccharides from *Ganoderma lucidum* can be gained as follows: infiltration time, microwave power density, and VM radiation time were 69.6 min, 11.22 W/g, and 180.54 s, respectively. Conducted in these optimal radiation pretreatment process conditions, the extraction yield of polysaccharide could reach up to 1.780%. Considering the operating conditions, the optimal pretreatment parameters for VM radiation extraction of *Ganoderma lucidum* polysaccharides could be revised as follows: infiltrating time, 70 min; microwave power density, 11.2 W/g; VM radiation time, 180 s. Accordingly, actual measurement for extraction yield of polysaccharides was 1.775%. Compared with theory predictions, the relative error was around 2.8%.

*3.3. Comparison with Traditional Hot-Water Bath Extraction.* Table 5 showed the comparison of extraction yield between traditional hot-water bath extraction and VM radiation pretreatment extraction. The extraction yield was up to 1.775% when VM radiation was conducted with the above optimizing parameters. Compared to the traditional hot-water extraction method, VM pretreatment can shorten the extraction time by more than a half, and the polysaccharide extraction yield increased by 48.1%. The advantage of VM radiation pretreatment extraction over the traditional methods lies in a greener chemical process, including shorting extraction time, enhancing extract efficiency, and reducing the solvent use. It holds signification potential for further investigation, development, and application.

Table 5: Comparison of two extraction methods.

| Method of extraction | Extraction technology | Extraction yield (%) |
|---|---|---|
| Hot-water bath extraction | Solid/liquid rate, 1 : 15; extraction temperature, 80°C; extraction time, 150 min | 1.198 |
| VM radiation pretreatment + hot-water bath extraction | After VM pretreatment in optimal technological condition: solid/liquid rate, 1 : 15; extraction temperature, 80°C; extraction time, 70 min. | 1.775 |

## 4. Conclusions

Vacuum-microwave radiation pretreatment can accelerate the follow-up hot-water bath extraction polysaccharides from *Ganoderma lucidum*. It is efficient, energy-saving, and high-extraction yield.

Optimized technological parameters of infiltrating time, microwave power density, and VM radiation time are 70 min, 11.2 W/g, and 180 s, respectively, and the extraction yield of polysaccharides is 1.775%. Compared with the traditional hot-water bath extraction, the extraction yield improves 48.1% by this current combined method.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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