

# Optimization of Extraction Technology of Polysaccharide of *Tricholoma giganteum*

Meihua Mo\*, Shenghua Hu, Xuefeng Xu, Ziyang Ma, Yan Ni, Yaowu Wei, Jiang Nie

College of Food Science, South China Agricultural University, Guangzhou, China.  
Email: \*mindymo@163.com.

Received May 11<sup>th</sup>, 2013; revised June 11<sup>th</sup>, 2013; accepted July 15<sup>th</sup>, 2013

Copyright © 2013 Meihua Mo *et al.* This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

*Tricholoma giganteum* also being called *Tricholoma lobayense*, belongs to Basidiomycotina, Hymenomycetes, Agaricales, Tricholomataceae and Tricholoma. It has high economic and medicinal values and is a kind of rare edible mushroom. Modern pharmacological studies have shown that *T. giganteum* have antitumor, antioxidant, antimicrobial and free radicals scavenging activity, and it can inhibit high blood pressure and HIV-1 reverse transcriptase. In this study, the technologies of isolation and purification of polysaccharides of *T. giganteum* were systematically studied. *T. giganteum* polysaccharides were obtained by hot-water extraction method. The infection of the extraction temperature, the extraction time, the solvent-solid ratio and the volume of solvent used during precipitation on the polysaccharides extraction ratio were studied. The extraction process was optimized by the uniform design (UD) method on the basis of the results of the single-factor experiments. The optimum extraction conditions of the mushroom polysaccharides (MP) were as follows: Extraction temperature was 100°C; extraction time was 3 hours; solvent-solid ratio was 20:1 and 5 times volume of 95% ethanol. The yield of polysaccharides of *T. giganteum* was 12.92%.

**Keywords:** Polysaccharide; *Tricholoma giganteum*; Extraction; Uniform Design

## 1. Introduction

*Tricholoma giganteum*, also known as *T. lobayene* and *Macrocybe giganteum*, distributes in the pantropical regions such as Asia, Africa, etc. and mainly distributes in USA, Japan, China, India, and Korean etc. [1-5]. It belongs to Basidiomycotina, Hymenomycetes, Agaricales, Tricholomataceae, Tricholoma. It has high economic and medicinal values. Its fruit bodies are rich in protein, polysaccharides, dietary fiber, mineral salts, vitamins and other healthful substances [6]. Modern pharmacological studies have shown that *T. giganteum* have antitumor, antioxidant, antimicrobial and free radicals scavenging activity. It has a novel angiotensin I-converting enzyme inhibitory peptide and can inhibit HIV-1 reverse transcriptase [7-15]. *T. giganteum* has received considerable attention in recent years because of the nutritional and health protective value.

Macromolecular compounds with important biological function. MP has the biological effects of detoxification, anti-oxidation, reducing blood pressure, reducing blood lipids, enhancing immune and lowering cholesterol levels

[9,16-20]. Currently, the extraction methods of mushroom polysaccharides (MP) mainly include hot water extraction, ultrasonic extraction, enzyme extraction, alkaline extraction, acid extraction and microwave extraction [16,21-23]. Ultrasonic extraction technology has been widely used in the extraction of plant active ingredients. The main mechanism of ultrasonic extraction is due to cavitation generated by ultrasound [24]. The advantage is to greatly increase the extraction yield of active ingredients, reduce the extraction time and avoid the destruction of the active ingredients [25]. But it is not suitable for practical application because of its high cost. Enzyme extraction can hydrolyze the cellulose, pectin and crude protein, and break down the cell walls. It is a more advanced and effective extraction method [26]. Water extraction is the traditional method of polysaccharide. This method is simple. But the parameters of its extraction only remain in the single factor tests and ignore the process of MP interaction between the factors. The extraction of polysaccharides from mushroom may be affected by various factors, such as the extraction temperature, the extraction time, the solvent-solid ratio and the volume of solvent used during precipitation. When

\*Corresponding author.

many factors and interactions affect the desired result, the uniform design (UD) method is an effective tool for finding their optimal values. UD is a statistical-mathematical method which uses quantitative data in an experimental design to determine and to solve multivariable equations in order to optimize processes or products. It was proposed by Fang [27], based on quasi-Monte Carlo method or number-theoretic method. It is precisely such a technique for experimental designs, emphasizing the uniformity of space filling in experimental domain. More recently, theoretical results from the relationship between the uniformity of the experimental points in the domain and some advanced properties of experimental designs have also provided strong support to this concept. One can even simply use uniformity as a criterion to obtain better orthogonal designs. These processes are affected by numerous variables and it is necessary to select those that have major effects as well as to identify their levels. It is widely applied for different purposes in chemical and biochemical processes [27-29]. The aim of the present work is to study the effects of the temperature of extraction, the time of extraction, the solvent-solid ratio and the volume of solvent used during precipitation on the MP extraction ratio as well as to optimize extraction conditions by using the UD.

## 2. Materials and Methods

### 2.1. *Tricholoma giganteum*

*Tricholoma giganteum* was provided by Guangzhou Yuewang Agricultural Limited Company, Guangzhou City, Guangdong Province, China.

### 2.2. Preparation of Mushroom Powder

The fruit-bodies of *T. giganteum* were collected from the mushroom plant and were cleaned. The mushroom head was cut. The rest of the fruit-bodies were washed and dried at 50°C, then ground in a grinder and sieved with 80 mesh. The mushroom powder was kept at the temperature of 4°C.

### 2.3. Extraction of Polysaccharides

One gram of mushroom powder was weighed for different optimization tests including extraction temperature, extraction time, solvent-solid ratio, Crude water-soluble polysaccharides were extracted with pure hot water and precipitated with 95% ethanol, yielding the MP. The extraction was dried to calculate the yield.

### 2.4. Experimental Design and Statistical Analysis

The effect of the four variables at five variation levels (Table 1) on MP extraction ratio was studied to determine the optimum combination of the variables, using

the  $U_5$  ( $5^4$ ) uniform design table (Table 2) to arrange the experiment (Table 3).

The model proposed for the target function  $Y$  (extraction ratio) is given below:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_1^2 + b_6x_2^2 + b_7x_3^2 + b_8x_4^2 + b_9x_1x_2 + b_{10}x_1x_3 + b_{11}x_1x_4 + b_{12}x_2x_3 + b_{13}x_2x_4 + b_{14}x_3x_4 \quad (1)$$

where  $b_0$  was offset term,  $b_1, b_2, b_3$  and  $b_4$  were related to the linear effect terms,  $b_5, b_6, b_7$ , and  $b_8$  were connected to the quadratic effects and  $b_9, b_{10}, b_{11}, b_{12}, b_{13}$  and  $b_{14}$  were associated with the interaction effects,  $x_1, x_2, x_3, x_4$  were the four variables.

The adequacy of the polynomial model was expressed by the multiple coefficient of determination,  $R^2$ . The significance of each coefficient was determined by using F

**Table 1. Levels of the variables tested in the Uniform design.**

Levels No.	Levels of the variables			
	Extraction temperature (°C)	Extraction time (h)	solvent-solid Ratio (ml/g)	the volume ratio of 95% ethanol (ml/mg)
	$x_1$	$x_2$	$x_3$	$x_4$
1	80	3	20:1	3:1
2	85	4	30:1	4:1
3	90	5	40:1	5:1
4	95	6	50:1	6:1
5	100	7	60:1	7:1

**Table 2.  $U_5$  ( $5^4$ ) Uniform design table.**

Tests	Levels No.			
	$x_1$	$x_2$	$x_3$	$x_4$
1	1	2	3	4
2	2	4	1	3
3	3	1	4	2
4	4	3	2	1
5	5	5	5	5

**Table 3. Experimental scheme of UD and results.**

Tests No.	Levels of the variables				Extraction ratio (%) $Y$
	Extraction temperature (°C)	Extraction time (h)	solvent-solid ratio (ml/g)	Volume of 95% ethanol	
	$x_1$	$x_2$	$x_3$	$x_4$	
1	80	4	40:1	6:1	10.55
2	85	6	20:1	5:1	10.93
3	90	3	50:1	4:1	11.90
4	95	5	30:1	3:1	12.34
5	100	7	60:1	7:1	11.92

value and P value. The optimum condition was verified by conducting experiments under these conditions. The result was compared with the model predictions and the test which the extraction ratio was the highest in **Table 3**. Statistical analysis was performed by using the DPS data processing system [30].

### 3. Results and Discussion

#### 3.1. Results of UD

The scheme and results of MP extraction are listed in the last column in **Table 3**. The data were analyzed using the DPS data processing system. It is suggested that a quadratic regression analysis be used to identify the significant variables, relationship coefficient and the model parameters. The model can be used to gain the optimum conditions of the extraction process and predict the MP extraction ratio [28].

The regression equation representing the relationship between MP extraction ratio and the test variables derived from UD were as follows:

$$Y = 2.8932 + 0.1039x_1 - 0.0003x_2x_3 - 0.0254x_2x_4 \quad (2)$$

$Y$  is a coded fitting equation.  $x_1$ ,  $x_2$ ,  $x_3$ ,  $x_4$  were the four variables. When the  $p$  value is less than 0.05, it shows that this term is significant, on the other hand, when the  $p$  value is less than 0.01, it shows that the term is highly significant and that means this term had a greater influence than other variables [13].  $F$  and  $p$  value, which determines the significance of each coefficient. The multiple coefficient of correlation ( $R = 0.99996$ ) and the modified coefficient ( $R_a = 0.99994$ ) indicated high agreement between experimental and predicted values of the MP extraction ratio in this experiment. The  $p$  value was 0.0018 and less than 0.01,  $F$  value was 166666.4. This proved that the regression equation was reliable.

The corresponding variables will be more significant if the absolute  $t$  value becomes larger and  $p$  value becomes smaller. According to the regression terms coefficient  $t$  analysis result (**Table 4**) indicated that the most significant variable was linear the temperature of extraction ( $x_1$ ) and the interaction between the time of extraction ( $x_2$ ) and Solid-liquid ratio ( $x_3$ ). Their  $p$  values were 0.0001 which seem highly significant. That was followed by the interaction between the time of extraction ( $x_2$ ) and Volume ratio of 95% ethanol ( $x_4$ ). The  $p$  value was 0.0106 and less than 0.05, which seem significant. According  $F$  and  $p$  values other terms of  $b_2$ ,  $b_3$ ,  $b_4$ ,  $b_5$ ,  $b_6$ ,  $b_7$ ,  $b_8$ ,  $b_9$ ,  $b_{10}$ ,  $b_{11}$  and  $b_{14}$  did not have the statistical significance.

The experimental values, predicted values and error of fitting were listed in **Table 5**. The biggest error of fitting was just 0.0017, it means that the regression equation fit very well.

Mathematic model simulation analysis was conducted to find out the maximum extraction ratio of MP (12.92%)

**Table 4. Regression terms coefficient  $t$  analysis result.**

Regression terms	Partial Correlation Coefficient	$t$ value	Level of Significance ( $p$ )
$x_1$	1	377.8397	0.0001
$x_2 \times x_3$	-0.9947	9.6466	0.0106
$x_2 \times x_4$	-1	133.9125	0.0001

**Table 5. Experimental values, predicted values and error of fitting.**

Tests No.	Experimental values	Predicted values	Error of fitting
1	10.55	10.5510	-0.0010
2	10.93	10.9293	0.0007
3	11.90	11.8983	0.0017
4	12.34	12.3415	-0.0015
5	11.92	11.9199	0.0001

was obtained when the temperature is 100°C, the time is 3 hours, solvent-solid ratio 20:1, precipitated with 5 times volume of 95% ethanol.

#### 3.2. Verification of Results

In order to examine whether the equation gained from UD could fit the relationship between extraction ratio and each variable well, confirmatory experiment was carried out. The extraction ratio gained from the optimal extraction conditions reached 12.92%, comparing to the predicted extraction ratio 13.00% was not different significantly ( $p > 0.05$ ) and comparing to the extraction ratio 12.35% gained from test No. 4 in **Table 3**, they are different significantly ( $p < 0.05$ ). This result showed that the technology of MP extraction obtained from UD was accurate and reliable.

### 4. Concluding Remarks

The present study had shown that the extraction ratio of the MP was optimized by using UD. The four independent variables, involved in the optimization, were the temperature of extraction, the time of extraction, the solvent-solid ratio and the volume of 95% ethanol in precipitation. The UD result indicated that the variable with the largest effect was the temperature of extraction and the interaction between the time of extraction ( $x_2$ ) and the solvent-solid ratio ( $x_3$ ). That was followed by the interaction between the time of extraction ( $x_2$ ) and the volume of 95% ethanol ( $x_4$ ). Moreover, the optimal MP yield of 0.1292 g from 1 g mushroom powder of *T. giganteum* was obtained.

### 5. Acknowledgements

We gratefully acknowledge the financial support received

ed in the form of a research Grant (Grant No. 31272218) from the National Natural Science Foundation of China; the Planned Scientific and Technological Project of Guangdong Province (Grant No. 2011B020- 303007); The Applied Basic Research Foundation of Guangzhou (Grant No. 2012J4100125); The national Spark Program project (Grant No. 2012GA780042); and Guangdong Province Agricultural Standards (Amendment) and standardization research project.

## REFERENCES

- [1] S. T. Chang and X. L. Mao, "Hong Kong Mushrooms," The Chinese University of Hong Kong Press, Hong Kong, 1995.
- [2] C. Y. Guo and Y. F. Shen, "Isolation and Domestication of Strain 'Xia-3' from *Tricholoma giganteum*," *Subtropical Plant Science*, Vol. 31, No.1, 2002, pp. 14-16.
- [3] H. K. Kim, Y. S. Kim, S. J. Seok, G. P. Kim and D. Y. Cha, "Artificial Cultivation of *Tricholoma giganteum* Collected in Korea (I)-Morphological Characteristics of Fruitbody and Environmental Condition in Habitat of *T. giganteum*," *Korean Journal of Mycology*, Vol. 26, No. 2, 1998, pp. 182-186.
- [4] Y. L. Liu, S. M. Tan, M. Y. Wun and E. Liang, "A Study on the Isolation and Identification of Wild *Tricholoma lobayense* Strain," *Acta Edulis Fung*, Vol. 8, No. 2, 2001, pp. 19-23.
- [5] M. Yoshikazy and T. Mizuno, "Cultivation of Niohshimeji (*Tricholoma giganteum*)," *Food Reviews International*, Vol. 13, No. 3, 1997, pp. 413-418. [doi:10.1080/87559129709541127](https://doi.org/10.1080/87559129709541127)
- [6] Y. Z. Wang, H. M. Y. H. Tang and Z. F. Zhang, "Analysis of Main Nutrition Components in *Tricholoma giganteum* Fruitbodies," *Acta Edulis Fungi*, Vol. 12, No. 2, 2005, pp. 24-26.
- [7] Y. X. Guo, H. X. Wang and T. B. Ng, "Isolation of Trichogin: An Antifungal Protein from Fresh Fruiting Bodies of the Edible Mushroom *Tricholoma giganteum*," *Peptides*, Vol. 26, No. 4, 2005, pp. 575-580. [doi:10.1016/j.peptides.2004.11.009](https://doi.org/10.1016/j.peptides.2004.11.009)
- [8] D. H. Lee, J. H. Kim, J. S. Park, *et al.*, "Isolation and Characterization of a Novel Angiotensin I-Converting Enzyme Inhibitory Peptide Derived from the Edible Mushroom *Tricholoma giganteum*," *Peptides*, Vol. 25, No. 4, 2004, pp. 621-627. [doi:10.1016/j.peptides.2004.01.015](https://doi.org/10.1016/j.peptides.2004.01.015)
- [9] F. Liu, V. E. C. Ooi and S. T. Chang, "Free Radicals Scavenging Activity of Mushroom Polysaccharide Extracts," *Life Science*, Vol. 60, No. 10, 1997, pp. 763-771. [doi:10.1016/S0024-3205\(97\)00004-0](https://doi.org/10.1016/S0024-3205(97)00004-0)
- [10] F. Liu, V. E. C. Ooi, W. K. Liu and S. T. Chang, "Immuno-Modulation and Antitumor Activity of Polysaccharide-protein Complex from the Culture Filtrates of a Local Edible Mushroom, *Tricholoma lobayense*," *Mycosystema*, Vol. 27, No. 4, 1996, pp. 621-624. [doi:10.1016/0306-3623\(95\)02058-6](https://doi.org/10.1016/0306-3623(95)02058-6)
- [11] J. L. Mau, H. C. Lin and S. F. Song, "Antioxidant Properties of Several Specially Mushroom," *Food Research International*, Vol. 35, No. 6, 2002, pp. 519-526. [doi:10.1016/S0963-9969\(01\)00150-8](https://doi.org/10.1016/S0963-9969(01)00150-8)
- [12] T. Mizuno, T. Kinoshita, C. Zhang, H. Ito and Y. Mayuzum, "Antitumor-Active Heteroglycans from Niohshimeji Mushroom, *Tricholoma giganteum*," *Bioscience, Biotechnology, and Biochemistry*, Vol. 59, No. 4, 1995, pp. 568-571. [doi:10.1271/bbb.59.568](https://doi.org/10.1271/bbb.59.568)
- [13] M. H. Mo and Q. M. Zhang, "Studies on Antimicrobial Activity of the Extracts of *Macrocybe gigantean*," *Science and Technology of Food Industry*, Vol. 30, No.5, 2009, pp. 151-153.
- [14] H. X. Wang and T. B. Ng, "Purification of a Novel Low-Molecular-Mass Laccase with HIV-1 Reverse Transcriptase Inhibitory Activity from the Mushroom *Tricholoma giganteum*," *Biochemical and Biophysical Research Communications*, Vol. 315, No. 2, 2004, pp. 450-454. [doi:10.1016/j.bbrc.2004.01.064](https://doi.org/10.1016/j.bbrc.2004.01.064)
- [15] Q. M. Zhang and M. H. MO, "Study of Chemical Components and Antimicrobial Activity of Volatile Oil of *Macrocybe gigantean*," *Modern Food Science and Technology*, Vol. 24, No. 12, 2008, pp. 1232-1235.
- [16] Y. H. Jiang, X. L. Jiang, P. Wang, H. J. Mou, X. K. Hu and S. Q. Liu, "The Antitumor and Antioxidative Activities of Polysaccharides Isolated from *Isaria farinosa* BOS," *Microbiological Research*, Vol. 163, No. 4, 2008, pp. 424-430. [doi:10.1016/j.micres.2006.07.002](https://doi.org/10.1016/j.micres.2006.07.002)
- [17] H. Nanba, N. Kodama and D. Schar, "Effects of Maitake (*Grifola frondosa*) Glucanin HIV Infected Patients," *Mycoscience*, Vol. 41, No. 4, 2000, pp. 293-295. [doi:10.1007/BF02463941](https://doi.org/10.1007/BF02463941)
- [18] W. L. Shi, G. Z. Chen, H. Han, X. Chen, Y. K. Hong, L. K. Chen, D. Chen, *et al.*, "Extraction, Characterization of the Polysaccharide Extracts from Se-enriched *G. lucidum* (Se-GLP) and Its Inhibition Against Oxidative Damage in Ischemic Reperfusion Mice (vol. 80, pg 774, 2010)," *Carbohydrate Polymers*, Vol. 83, No. 3, 2011, pp. 1416-1416. [doi:10.1016/j.carbpol.2010.07.001](https://doi.org/10.1016/j.carbpol.2010.07.001)
- [19] C. Y. Yan, Y. Yin, D. W. Zhang, W. Yang and R. M. Yu, "Structural Characterization and *in Vitro* Antitumor Activity of a Novel Polysaccharide from *Taxus yunnanensis*," *Carbohydrate Polymers*, Vol. 96, No. 2, 2013, pp. 389-395. [doi:10.1016/j.carbpol.2013.04.012](https://doi.org/10.1016/j.carbpol.2013.04.012)
- [20] C. Zou, Y. M. Du, Y. Li, J. H. Yang, T. Feng, L. Zhang, *et al.*, "Preparation of Lacquer Polysaccharide Sulfates and Their Antioxidant Activity *in Vitro*," *Carbohydrate Polymers*, Vol. 73, No. 2, 2008, pp. 322-331. [doi:10.1016/j.carbpol.2007.11.035](https://doi.org/10.1016/j.carbpol.2007.11.035)
- [21] P. Ghosh, P. Ghosal, S. Thakur, P. Lerouge, C. Loutelier-Bourhis, A. Driouch, *et al.*, "Polysaccharide from *Sesamum indicum* Meal: Isolation and Structural Features," *Food chemistry*, Vol. 90, No. 4, 2005, pp. 719-726. [doi:10.1016/j.foodchem.2004.04.032](https://doi.org/10.1016/j.foodchem.2004.04.032)
- [22] S. Q. Huang and Z. X. Ning, "Extraction of Polysaccharide from *Ganoderma lucidum* and Its Immune Enhancement Activity," *International Journal of Biological Macromolecules*, Vol. 47, No. 3, 2010, pp. 336-341. [doi:10.1016/j.ijbiomac.2010.03.019](https://doi.org/10.1016/j.ijbiomac.2010.03.019)
- [23] R. M. Yu, Y. Yin, *et al.*, "Structural Elucidation and Bio-

- logical Activity of a Novel Polysaccharide by Alkaline Extraction from Cultured *Cordyceps militaris*,” *Carbohydrate Polymers*, Vol. 75, No. 1, 2009, pp. 166-171. [doi:10.1016/j.carbpol.2008.07.023](https://doi.org/10.1016/j.carbpol.2008.07.023)
- [24] Z. Hromadkova, A. Ebringerova and P. Valachovic, “Ultrasound-Assisted Extraction of Water-Soluble Polysaccharides from the Roots of Valerian (*Valeriana officinalis* L.),” *Ultrasonics Sonochemistry*, Vol. 9, No. 1, 2008, pp. 37-44. [doi:10.1016/S1350-4177\(01\)00093-1](https://doi.org/10.1016/S1350-4177(01)00093-1)
- [25] S. Q. Huang, J. W. Li, Z. Wang, H. X. Pan, J. X. Chen and Z. X. Ning, “Optimization of Alkaline Extraction of Polysaccharides from *Ganoderma lucidum* and Their Effect on Immune Function in Mice,” *Molecules*, Vol. 15, No. 5, 2010, pp. 3694-3708. [doi:10.3390/molecules15053694](https://doi.org/10.3390/molecules15053694)
- [26] Z. H. Jiang, X. L. Yin, Q. H. You and S. Y. Zheng, “Studies on Extraction of *Tricholoma matsutake* Polysaccharides by Complex Enzyme and Molecular Weight Distribution,” *Acta Edulis Fung*, 2010, pp. 109-112.
- [27] K. T. Fang and H. Qin, “A Note on Construction of Nearly Uniform Designs with Large Number of Runs,” *Statistics & Probability Letters*, Vol. 61, No. 2, 2003, pp. 215-224. [doi:10.1016/S0167-7152\(02\)00357-7](https://doi.org/10.1016/S0167-7152(02)00357-7)
- [28] Y. Z. Liang, K. T. Fang and Q. S. Xu, “Uniform Design and Its Applications in Chemistry and Chemical Engineering,” *Chemometrics and Intelligent Laboratory Systems*, Vol. 58, 2001, pp. 43-57.
- [29] Y. Y. Tan, Y. C. Jiao, H. Li and X. K. Wang, “MOEA/D Plus Uniform Design: A New Version of MOEA/D for Optimization Problems with Many Objectives,” *Computers & Operations Research*, Vol. 40, No. 6, 2013, pp. 1648-1660. [doi:10.1016/j.cor.2012.01.001](https://doi.org/10.1016/j.cor.2012.01.001)
- [30] Q. Y. Tang and C. X. Zhang, “Data Processing System (DPS) Software with Experimental Design, Statistical Analysis and Data Mining Developed for Use in Entomological Research,” *Insect Science*, Vol. 20, No. 2, 2013, pp. 256-260. [doi:10.1111/j.1744-7917.2012.01519.x](https://doi.org/10.1111/j.1744-7917.2012.01519.x)