DOI: http://dx.doi.org/10.1590/1678-457X.00916

Homogenate extraction of crude polysaccharides from *Lentinus edodes* and evaluation of the antioxidant activity

Leqin KE[1]*, Haiyan CHEN[1]

Abstract

Crude polysaccharides of *Lentinus edodes* were extracted using homogenate method. Factors affecting the yield of crude polysaccharides were investigated and optimized by response surface methodology. The homogenate extraction method was compared with traditional heating extraction method. The antioxidant activity of crude polysaccharides from *Lentinus edodes* was evaluated. Results showed that, the optimal conditions of homogenate extraction were as follows: solvent pH, 10; liquid-solid ratio, 30: 1 (mL: g), extraction time, 66 s; number of extraction, 1. Under these conditions, the yield of crude polysaccharides was (13.2 ± 0.9)%, which was 29.82% higher than that of traditional heating extraction. Crude polysaccharides of *Lentinus edodes* had good DPPH scavenging activity. Compared with the traditional heating extraction, the homogenate extraction had notable advantages including good extraction yield, short extraction time and low extraction temperature. It is an efficient way to extract crude polysaccharides from *Lentinus edodes*.

Keywords: crude polysaccharides; *Lentinus edodes*; homogenate extraction; response surface methodology.

Practical Application: The homogenate extraction method can be applied to extracting crude polysaccharides from *Lentinus edodes*.

1 Introduction

Edible mushrooms are rich in dietary fiber, minerals, vitamins and low in fat (Manzi et al., 2001), and have long been used in folk medicines and health foods. *Lentinus edodes*, also named *Xianggu* in Chinese and Shiitake in Japanese, is one of the most widely edible mushrooms on the global market due to its taste and nutritional values (Hatvani & Mécs, 2001). Crude polysaccharides are the most active constituents in *Lentinus edodes*. Modern medical research finds that, *Lentinus edodes* polysaccharides are a kind of host defense potentiator, which can increase the number of lymphocytes in body (Ahmed et al., 2011), inhibit the allogeneic, syngeneic and primary tumors (Zakany et al., 1980), and prevent carcinogenesis induced by chemical factors or virus (Suga et al., 1984). At the same time, *Lentinus edodes* polysaccharides can enhance the resistance of host to different bacteria, parasitic diseases and virus including HIV (Lapis et al., 1989), and reduce the toxicity reaction of radiotherapy and chemotherapy (Wang & Gong, 2001). Mushrooms contain various compounds such as polyphenols and crude polysaccharides, which are recognized as excellent antioxidants (Palacios et al., 2011; Thetsrimuang et al., 2011; He et al., 2012). Studying the antioxidant capacity of mushrooms has begun to take the spotlight in recent researches (Zheng et al., 2005; Sun et al., 2008; Liu et al., 2010; Qiao et al., 2010).

Extraction of crude *Lentinus edodes* polysaccharides is conventionally performed by traditional heating method using water, which is laborious and time-consuming, and requires large amounts of solvent. Homogenate extraction is an alternative to conventional extraction methods, through which the chemical compositions are extracted from materials in solvent by high-speed mechanical shearing, mixing, cutting and smashing without heating and pressure. This method has been proved to be effective to extract gardenia yellow pigment from *Gardenia Jasminoides Ellis* fruit (Zhu et al., 2014), camptothecine and hydroxylcamptothecin from Camptotheca acuminata leaves (Shi et al., 2009), and isoflavones from soybean meal (Zhu et al., 2011). However, application of homogenate extraction to extracting crude polysaccharides from *Lentinus edodes* has never been reported. In this study, the homogenate extraction method was applied to extraction of crude polysaccharides from *Lentinus edodes*. Effects of various extraction parameters such as solvent pH, liquid-solid ratio, extraction time and number of extraction were investigated. The homogenate extraction method was compared with traditional heating extraction method, and the antioxidant activity of crude *Lentinus edodes* polysaccharides was investigated.

2 Materials and methods

2.1 Materials

Lentinus edodes was derived from Lishui City, Zhejiang Province, China. They were dried at 60 °C for 6 h (final moisture content, 5%), pulverized into to a certain particle size (average diameter, 2 mm) by a disintegrator, and kept in dry place

Received 13 Feb., 2016
Accepted 15 June, 2016
[1] *College of Ecology, Lishui University, Lishui, Zhejiang Province, China*
*Corresponding author: keleqin12@163.com

until used. Homogenate extractor (JHBE-50A) was provided by Golden Star Technology, Inc., Ltd. (Zhengzhou, China) (Figure 1). Glucose standard was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Other analytical-grade reagents were purchased from Jiangsu Feiya Chemical Industry Co., Ltd. (Nantong, China).

2.2 Process of homogenate extraction

Lentinus edodes (20.0 g) and alkaline water (pH was adjusted with NaOH) were put into the homogenate extractor (motor voltage, 200 V) for extraction with suitable solvent pH, liquid-solid ratio, extraction time and number of extraction at room temperature (20-25 °C). After extraction, the mixture was centrifuged at 4500 rpm for 10 min. The supernatant was collected and concentrated by rotary evaporator. Concentrated solution was precipitated by adding fourfold volume of anhydrous ethanol and then incubated at 5 °C for 10 h. After centrifugation and vacuum drying, the mixture was preliminarily purified according to the reported method (You et al., 2014). Finally, the crude polysaccharides were obtained. Each experimental group was repeated for three times. The yield of crude polysaccharides was expressed *as* Yield (%) = (m / M)*100%, where m was weight of crude polysaccharides analyzed by phenol-sulfuric acid method (g), and M was weight of *Lentinus edodes* (g).

Figure 1. Homogenate extractor (JHBE-50A).

2.3 Process of traditional heating extraction

Under optimized conditions, *Lentinus edodes* (20.0 g) and water were put into a reflux apparatus for extraction at 80 °C for 2 h. Extracting solution was collected together and centrifuged (4500 rpm, 10 min). Latter steps were the same as homogenate extraction. The whole extraction process was carried out thrice.

2.4 Identification of crude polysaccharides

Crude polysaccharides were identified using modified phenol-sulfuric acid method (Dubois et al., 1956). The crude polysaccharides solution was mixed with phenol and concentrated sulfuric acid to reach color, and the light absorption was recorded at 490 nm using UV spectrophotometer.

2.5 Determination of antioxidant activity

The antioxidant activity of crude *Lentinus edodes* polysaccharides obtained under optimal conditions was determined by 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, according to the method reported by Mau et al. (2002) with some modifications. 2.0 mL of crude polysaccharides solution with various concentrations (100-5000 mg/L) was mixed with equal volume of alcoholic solution of DPPH (2.0×10^{-4} mol/L). The reaction mixture was shaken vigorously and incubated for 30 min at room temperature in a dark place, and then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The scavenging activity (SA) of crude polysaccharides on DPPH was calculated according to equation: SA% = [1-(A_i-A_j) / A_0] × 100%, where A_i was absorbance of the reaction mixture, A_j was absorbance of the reaction mixture without DPPH, A_0 was absorbance of reaction mixture without crude polysaccharides. The test process was carried out thrice, and the results were presented as mean±SD.

2.6 Experiment design

Response surface methodology (RSM) is an empirical modeling technique used to estimate relationships between a set of experimental control factors and observed results. In this study, Box-Benhnken design (BBD) was applied to optimize extraction conditions by using software Design-Expert (Trial Version 8.0.6, Stat-Ease Inc., Minneapolis, MN, USA). According to preliminary experiment results, solvent pH, liquid-solid ratio

and extraction time were chosen as key parameters and were designated as Z_1, Z_2 and X_3, respectively. After conversion, the variables for BBD were obtained as follows: $X_1 = (Z_1 - 10)/1$; $X_2 = (Z_2 - 30)/10$; $X_3 = (Z_3 - 60)/20$. Ranges of variables and their levels were shown in Table 1.

2.7 Statistical analysis

Data were presented as mean±SD. Optimization of extraction parameters was performed by response surface methodology using Design-Expert 8.0 software (Stat-Ease, Inc., MN, USA).

Table 1. Independent variables and their levels for BBD.

Variable	Level		
	-1	0	1
Solvent pH (X_1)	9	10	11
Liquid-solid ratio (mL: g) (X_2)	20:1	30:1	40:1
Extraction time (s) (X_3)	40	60	80

Comparisons between two groups were performed by t test, using SPSS17.0 software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered as statistically significant.

3 Results and discussion

3.1 Preliminary experiments

There were many factors affecting the yield of crude polysaccharides in homogenate extraction, among which solvent pH, liquid-solid ratio (mL: g), extraction time (s) and number of extraction were relatively important. Effects of these factors on yield of crude polysaccharides were firstly investigated by single factor experiments. Results were shown in Figure 2. Solvent pH of 10, liquid-solid ratio of 30: 1 (mL: g), extraction time of 60 s, and number of extraction of 3 were the optimal value of each factor. However, the increase extent of crude polysaccharides yield with increase of number of extraction was not obvious. Therefore, the effect of number of extraction on

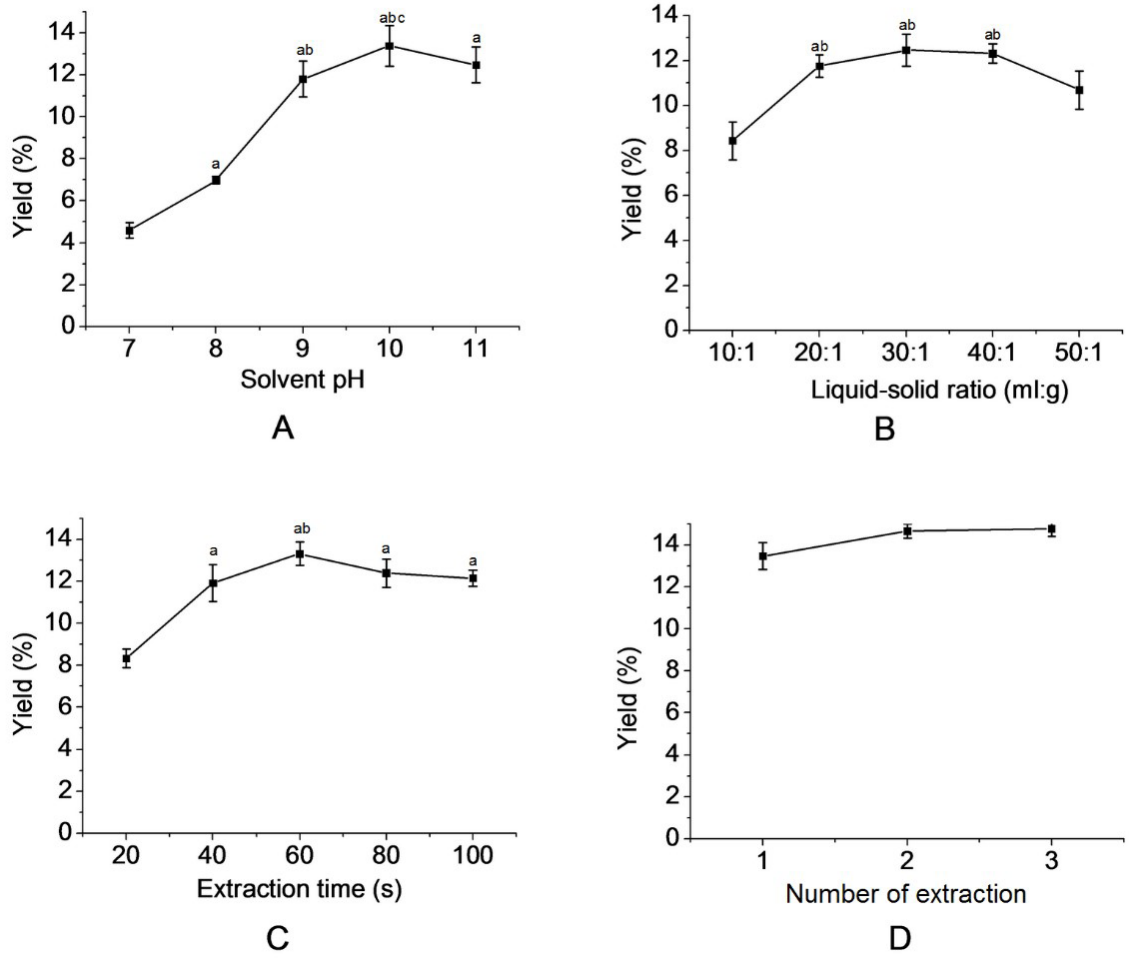

Figure 2. Effects of different operating factors on yield of crude polysaccharides in preliminary experiments. (A) solvent pH, $^aP < 0.05$ compared with pH 7 group, $^bP < 0.05$ compared with pH 8 group, $^cP < 0.05$ compared with pH 9 group; (B) liquid-solid ratio, $^aP < 0.05$ compared with 10: 1 group, $^bP < 0.05$ compared with 50: 1 group; (C) extraction time, $^aP < 0.05$ compared with 20 s group, $^bP < 0.05$ compared with 40 s group; (D) number of extraction.

crude polysaccharides yield was not investigated in following optimization experiments. Considering extraction efficiency, number of extraction of 1 (once extraction) was enough for obtaining good yield of crude polysaccharides. In addition, in homogenate extraction, the extraction time was very short, and the effect of extraction temperature on the yield was not obvious. Therefore, we had not investigated the extraction temperature in homogenate extraction.

3.2 Optimization of homogenate extraction parameters by response surface methodology

According to the BBD, a total of 17 experimental runs were employed and experiments were performed in a randomized order. All experiments were repeated for three times. Results were shown in Table 2.

Optimization of homogenate extraction conditions was conducted by employing BBD. Data were analyzed using Design Expert 8.0.6 software for statistical analysis of variance (ANOVA), regression coefficients and regression equation. Polynomial equations, describing yield of crude polysaccharides (Y) as a simultaneous function of solvent pH (X_1), liquid-solid ratio (X_2), extraction time (X_3) were shown in equation: Y=13.05-2.19X_1-0.055X_2-1.43X_3-0.33X_1X_2-2.08X_1X_3-1.78X_2X_3-3.49X_1^2-4.47X_2^2-1.75X_3^2.

To evaluate the optimal extraction conditions of homogenate extraction for crude polysaccharides yield and relationship between response and significant variables, analysis of variance (ANOVA) for model was performed. As shown in Table 3, experimental data fitted well to quadratic models. ANOVA for response surface quadratic regression model showed that the model was highly significant ($P = 0.0002$) with a high F-value of 25.34. The order of factors affecting crude polysaccharides yield was as follows: solvent pH (X_1) > extraction time (X_3) > liquid-solid ratio (X_2). X_1, X_3, X_1^2, X_2^2 and X_1X_3 had highly significant effects on extraction yield ($P < 0.01$), and X_3^2 and X_2X_3 had significant effects on crude polysaccharides yield ($P < 0.05$).

Regression analysis of data showed that the coefficient of determination (R^2) value for crude polysaccharides was 0.9702, suggesting that the model was significant. The adjusted determination coefficient (Adj R^2 = 0.9319) was also satisfied to confirm the significance of model. At same time, the lack-of-fit statistics, which was used to test adequacy of model, indicated that the P value for crude polysaccharides yield (0.4474) was not significant. No abnormality was obtained from diagnoses of residual. Thus, it could be concluded that this model was statistically sound.

To depict the interactive effects of operational variables on responses, one variable was kept constant and other two variables varied in defined ranges. Response surfaces plots about crude polysaccharides yield were shown in Figure 3. Shapes of response surfaces and contour plots indicated the nature and extent of interaction between different variables. Solvent pH was the most significant factor affecting crude polysaccharides yield, representing a steep curved face, followed by extraction time. Liquid-solid ratio had little effect on extraction yield, of which the curved face was stepless. By solving inverse matrix, the conditions for maximum response value were obtained as follows: X_1 = -0.23,X_2 = 0.06,X_3 = -0.31. The optimal parameters were calculated as solvent pH of 9.77, liquid-solid ratio of 30.6: 1 (mL: g) and extraction time of 66.2 s. Under the optimal conditions, the predicted yield of crude polysaccharides was 13.5%.

3.3 Results of verification experiments

Adequacy of model equation for predicting optimal response values was tested using selected optimal extraction conditions. Accounting for the feasibility of experiments, the optimal extraction conditions were adjusted as follows: solvent pH, 10; liquid-solid ratio, 30: 1 (mL: g); extraction time, 66 s; once

Table 2. BBD and experimental results.

Run	Level			Variable			Yield of crude polysaccharides (%)
	X_1	X_2	X_3	Solvent pH	Liquid-solid ratio (mL: g)	Extraction time (s)	
1	-1	-1	0	9	20	60	7.5 ± 0.5
2	1	-1	0	11	20	60	3.5 ± 0.7
3	-1	1	0	9	40	60	7.4 ± 1.3
4	1	1	0	11	40	60	2.0 ± 0.7
5	-1	0	-1	9	30	40	8.7 ± 1.1
6	1	0	-1	11	30	40	8.8 ± 0.3
7	-1	0	1	9	30	80	11.0 ± 0.8
8	1	0	1	11	30	80	2.8 ± 0.6
9	0	-1	-1	10	20	40	6.7±1.0
10	0	1	-1	10	40	40	10.9 ± 0.8
11	0	-1	1	10	20	80	6.4 ± 1.2
12	0	1	1	10	40	80	3.4 ± 0.7
13	0	0	0	10	30	60	12.5 ± 0.4
14	0	0	0	10	30	60	12.3 ± 0.9
15	0	0	0	10	30	60	14.4 ± 0.5
16	0	0	0	10	30	60	13.9 ± 1.1
17	0	0	0	10	30	60	12.1 ± 0.8

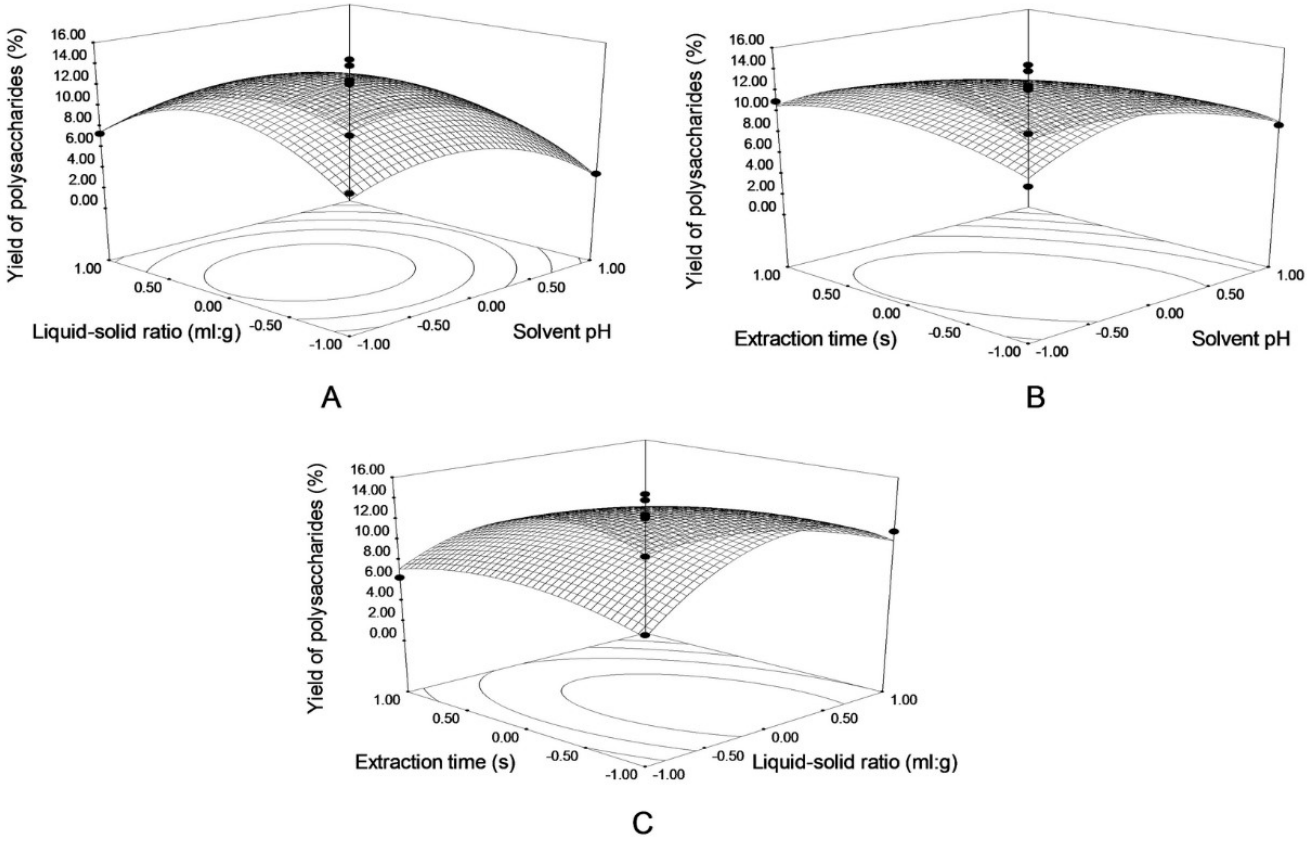

Figure 3. Response surface plots showing effects on yield of crude polysaccharides (A), solvent pH (X_1) and liquid-solid ratio (X_2); (B), solvent pH (X_1) and extraction time (X_3); (C) liquid-solid ratio (X_2) and extraction time (X_3).

Table 3. Analysis of variance of experimental results of BBD.

Source	Sum of Squares	df	Mean Square	F Value	Prob > F[a]
Model	248.16	9	27.57	25.34	0.0002
X_1	38.46	1	38.46	35.34	0.0006
X_2	0.024	1	0.024	0.022	0.8857
X_3	16.36	1	16.36	15.03	0.0061
X_1X_2	0.45	1	0.45	0.41	0.5412
X_1X_3	17.31	1	17.31	15.90	0.0053
X_2X_3	12.67	1	12.67	11.64	0.0113
X_1^2	51.23	1	51.23	47.07	0.0002
X_2^2	84.05	1	84.05	77.23	<0.0001
X_3^2	12.87	1	12.87	11.82	0.0109
Residual	7.62	7	1.09		
Lack of Fit	3.44	3	1.15	1.10	0.4474
Pure Error	4.18	4	1.05		
Cor Total	255.78	16	$R^2 = 0.9702$		

[a]$P < 0.05$ meant significant, and $P < 0.01$ meant highly significant.

extraction. The verification experiment was conducted and the yield of crude polysaccharides was (13.2 ± 0.9)%, which was not significant different from predicted value of 13.5%. Good correlation between them indicated that, this response model was adequate to reflect the expected optimization.

3.4 Comparison of homogenate extraction with traditional heating extraction

Homogenate extraction and traditional heating extraction under optimal conditions were compared for extraction of crude polysaccharides from *Lentinus edodes*. Results were shown in Table 4. The yield of crude polysaccharides using homogenate extraction was 29.4% higher than that in traditional heating extraction. Compared with traditional heating extraction method, the extraction time and number of extraction of homogenate extraction were significantly shortened. What's more, the homogenate extraction was performed at room temperature, which could save more energy.

3.5 Antioxidant activity of crude polysaccharides from Lentinus edodes

It is well accepted that scavenging of DPPH by antioxidants is attributable to their hydrogen donating activity (Chen & Ho, 1995). This test system can be used for primary characterization of antioxidant activity of compounds (Krings & Berger, 2001). The previous studies (Thetsrimuang et al., 2011; He et al., 2012) have reported that, the polysaccharides in mushrooms have antioxidant activity. As shown in Figure 4, crude *Lentinus edodes* polysaccharides had good DPPH scavenging activity, which was increased with the increasing concentration. The DPPH scavenging activity of crude polysaccharides by homogenate extraction was stronger than that by heating extraction at the

Table 4. Comparison between homogenate extraction and heating extraction.

Method	Extraction temperature	Extraction time	Solvent pH	Liquid-solid ratio (mL: g)	Number of extraction	Yield of crude polysaccharides (%)	*P*
A	Room temperature	66 s	10	30:1	1	13.2 ± 0.9	0.02
B	80 °C	2 h	9	30:1	2	10.2 ± 0.2	

A, homogenate extraction; B, heating extraction.

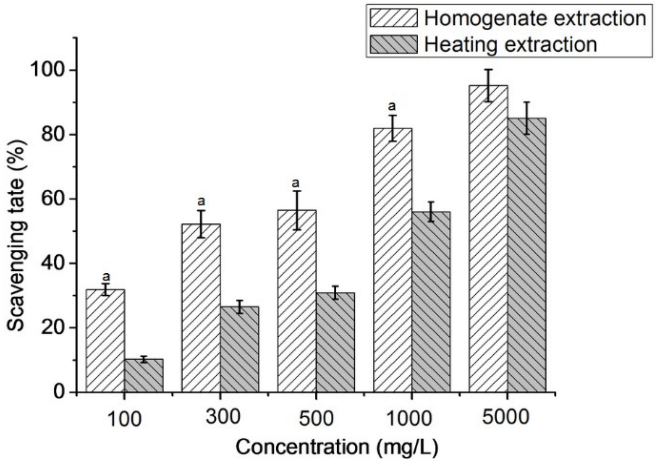

Figure 4. DPPH scavenging activity of *Lentinus edodes* crude polysaccharides by two extraction methods. $^{a}P < 0.05$ compared with heating extraction.

same concentration. This may be due to the loss of antioxidant components caused by high temperature in heating extraction.

In this study, we only investigated the antioxidant activity of extract obtained under the optimal conditions for each extraction method. In the future, the activity of extract under each extraction condition should be studied. There are many methods for determining the antioxidant activities of extracts, including ABTS, DPPH, FRAP, and ORAC assays (Dudonné et al., 2009). DPPH radical-scavenging assay is the common used method. In this study, due to condition limitation, we only used this representing method. This is also a limitation of the present study. In addition, due to complex compositions, the specific antioxidant components in crude polysaccharides of *Lentinus edodes* need to be confirmed by further experiments.

4 Conclusions

In the present study, homogenate extraction is used to extract crude polysaccharides from *Lentinus edodes*. The optimal extraction parameters are as follows: solvent pH, 10; liquid-solid ratio, 30: 1 (mL: g); extraction time, 66 s; number of extraction, 1. Under these conditions, the yield of crude polysaccharides is (13.2 ± 0.9)%. Compared with heating extraction method, the yield in homogenate extraction is higher, with shorter extraction time and lower temperature. The homogenate extraction method is an efficient way to extract crude polysaccharides from *Lentinus edodes*.

Acknowledgements

This work was supported by the Zhejiang Provincial Natural Science Foundation of China (No. LY16C150003; No. LQ14H290001; No. LY13H300003).

References

Ahmed, W. A., Osman, M. E., Khattab, O. H., Hassan, F. R. H., & El-Henawy, H. E. (2011). Studies of ethanolic extract from *Lentinus edodes* on different cell lines and lymphocytes separated from cancer breast patients. *Cancer Biology*, 1(2), 1-9.

Chen, C. W., & Ho, C. T. (1995). Antioxidant properties of polyphenols extracted from green and black teas. *Journal of Food Lipids*, 2(1), 35-46. http://dx.doi.org/10.1111/j.1745-4522.1995.tb00028.x.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for the determination of sugar and related substances. *Analytical Chemistry*, 28(3), 350-356. http://dx.doi.org/10.1021/ac60111a017.

Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., & Mérillon, J. M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, 57(5), 1768-1774. http://dx.doi.org/10.1021/jf803011r. PMid:19199445.

Hatvani, N., & Mécs, I. (2001). Production of laccase and manganese peroxidase by *Lentinus edodes* on malt-containing by-product of the brewing process. *Process Biochemistry*, 37(5), 491-496. http://dx.doi.org/10.1016/S0032-9592(01)00236-9.

He, J. Z., Ru, Q. M., Dong, D. D., & Sun, P. L. (2012). Chemical characteristics and antioxidant properties of crude water soluble polysaccharides from four common edible mushrooms. *Molecules (Basel, Switzerland)*, 17(4), 4373-4387. http://dx.doi.org/10.3390/molecules17044373. PMid:22495548.

Krings, U., & Berger, R. G. (2001). Antioxidant activity of some roasted foods. *Food Chemistry*, 72(2), 223-229. http://dx.doi.org/10.1016/S0308-8146(00)00226-0.

Lapis, K., Papay, J., Paku, S., & Szende, B. (1989). The effect of lentinan on the metastasis of Lewis lung carcinoma. *International Journal of Immunotherapy*, 5(4), 195-199.

Liu, J. C., Yue, L. L., Zhang, C., Fan, L., Zhou, L., Lin, Y., Niu, Y. C., Li, X. Y., Wen, X. C., & Sun, Y. X. (2010). A polysaccharide isolated from Agaricus blazei Murill inhibits sialyl Lewis X/E-selectin-mediated metastatic potential in HT-29 cells through down-regulating α-1,3-fucosyltransferase-VII (FucT-VII). *Carbohydrate Polymers*, 79(4), 921-926. http://dx.doi.org/10.1016/j.carbpol.2009.10.023.

Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, 73(3), 321-325. http://dx.doi.org/10.1016/S0308-8146(00)00304-6.

Mau, J. L., Lin, H. C., & Chen, C. C. (2002). Antioxidant properties of several medicinal mushrooms. *Journal of Agricultural and Food*

Chemistry, 50(21), 6072-6077. http://dx.doi.org/10.1021/jf0201273. PMid:12358482.

Palacios, I., Lozano, M., Moro, C., D'Arrigo, M., Rostagno, M. A., Martínez, J. A., García-Lafuente, A., Guillamón, E., & Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry*, 128(3), 674-678. http://dx.doi.org/10.1016/j.foodchem.2011.03.085.

Qiao, D., Liu, J., Ke, C. L., Sun, Y., Ye, H., & Zeng, X. X. (2010). Structural characterization of polysaccharides from *Hyriopsis cumingii. Carbohydrate Polymers*, 82(4), 1184-1190. http://dx.doi.org/10.1016/j.carbpol.2010.06.048.

Shi, W. G., Zu, Y. G., Zhao, C. J., & Yang, L. (2009). Homogenate extraction technology of camptothecine and hydroxycamptothecin from *Camptotheca acuminata* leaves. *Journal of Forest Research*, 20(2), 168-170. http://dx.doi.org/10.1007/s11676-009-0030-2.

Suga, T., Shiio, T., Maeda, Y. Y., & Chihara, G. (1984). Antitumor activity of lentinan in murine syngeneic and autochthonous hosts and its suppressive effect on 3-methylcholanthrene-induced carcinogenesis. *Cancer Research*, 44(11), 5132-5137. PMid:6488173.

Sun, Y., Wang, S., Li, T., Li, X., Jiao, L., & Zhang, L. (2008). Purification, structure and immunobiological activity of a new water-soluble polysaccharide from the mycelium of *Polyporus albicans* (Imaz.) Teng. *Bioresource Technology*, 99(4), 900-904. http://dx.doi.org/10.1016/j.biortech.2007.01.029. PMid:17368889.

Thetsrimuang, C., Khammuang, S., & Sarnthima, R. (2011). Antioxidant activity of crude polysaccharides from edible fresh and dry mushroom fruiting bodies of *Lentinus* sp. strain RJ-2. *International Journal of Pharmacology*, 7(1), 58-65. http://dx.doi.org/10.3923/ijp.2011.58.65.

Wang, J., & Gong, X. G. (2001). Polysaccharide of anti-tumor and immune regulation is reviewed. *Chinese Journal of Biochemical Pharmaceutics*, 22(2), 52-54.

You, Q., Yin, X., Zhao, Y., & Jiang, Z. (2014). Extraction and purification of polysaccharides from pine medicinal mushroom, *Tricholoma matsutake* (higher Basidiomycetes) fruit bodies. *International Journal of Medicinal Mushrooms*, 16(2), 149-160. http://dx.doi.org/10.1615/IntJMedMushr.v16.i2.50. PMid:24941036.

Zakany, J., Chihara, G., & Fachet, J. (1980). Effect of lentinan on tumor growth in murine allogeneic and syngeneic hosts. *International Journal of Cancer*, 25(3), 371-376. http://dx.doi.org/10.1002/ijc.2910250311. PMid:7390659.

Zheng, R., Jie, S., Hanchuan, D., & Moucheng, W. (2005). Characterization and immunomodulating activities of polysaccharide from *Lentinus edodes. International Immunopharmacology*, 5(5), 811-820. http://dx.doi.org/10.1016/j.intimp.2004.11.011. PMid:15778117.

Zhu, X. Y., Lin, H. M., Xie, J., Chen, S. S., & Wang, P. (2011). Homogenate extraction of isoflavones from soybean meal by orthogonal design. *Journal of Scientific and Industrial Research*, 70(6), 455-460.

Zhu, X. Y., Mang, Y. L., Shen, F. Q., Xie, J., & Su, W. K. (2014). Homogenate extraction of gardenia yellow pigment from *Gardenia Jasminoides Ellis* fruit using response surface methodology. *Journal of Food Science and Technology*, 51(8), 1575-1581. http://dx.doi.org/10.1007/s13197-012-0683-2. PMid:25114350.