Food Sci. Technol. Res., **17** (6), 461–470, 2011

Optimization of the Microwave-Assisted Extraction Process for Polysaccharides in Himematsutake (*Agaricus blazei Murrill*) and Evaluation of Their Antioxidant Activities

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Received April 6, 2011; Accepted August 3, 2011

Microwave-assisted extraction (MAE) technique was employed for the extraction of himematsutake (*Agaricus blazei Murrill*) polysaccharide (PMAE) and optimized by Box-Behnken design. The optimum conditions were extraction time 29.37 min, microwave power 400 W, extraction temperature 74.64°C and ratio of water to material 32.7:1 with an enhanced yield of 12.35%. Furthermore, PMAE had increased antioxidant activity in various oxidative systems in vitro when compared to polysaccharides extracted by conventional methods. The data obtained clearly showed that MAE is a fine way to extract the polysaccharides in *A. blazei Murrill*. Further, PMAE may be developed into a functional food as well as potential therapeutic agent.

Keywords: microwave-assisted extraction, polysaccharides, *Agaricus blazei Murrill*, antioxidant activity

Introduction

Reactive oxygen species (ROS) are inevitably generated during normal and/or aberrant consumption of molecular oxygen. These free radicals are able to damage numerous biological substances, including DNA, and exert some detrimental effects, which may even result in cell damage and chronic diseases (Dean *et al.*, 1993). Antioxidants may have an important role in the removal of these free radicals. However, the synthetic antioxidants most commonly used in industrial processing are suspected to be cytotoxic (Qi *et al.*, 2005; Valentao *et al.*, 2002). Thus, it is essential to develop effective, non-toxic and natural antioxidants that can protect the human body from free radicals and retard many chronic diseases (Kinsella *et al.*, 1993; Nandita and Rajini, 2004). In recent years, increasing evidence highlights that some polysaccharides isolated from plants have antioxidant activities (Sun *et al.*, 2009; Zou *et al.*, 2010; Fan *et al.*, 2009). Among various naturally substances, polysaccharides from some microorganisms may have antioxidant activity (Liu *et al.*, 2010).

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The polysaccharides of mushrooms including **himematsutake** (*Agaricus blazei Murrill*) have been reported to have numerous beneficial medicinal including potent antioxidant properties. However, the efficient extraction, the first step in the isolation of active polysaccharides is crucial. Successful extraction is influenced by the chemical nature of the polysaccharides, extraction method, storage time and conditions, and the presence of interfering substances. The conventional liquid-solid extraction techniques, such as heat reflux extraction (HRE), ultrasonic extraction (UE) and maceration extraction (ME) are discommodious, laborious, time-consuming and require large volumes of solvents. There is a need for development of more efficient and rapid extraction methods of polysaccharides of mushrooms.

Microwave-assisted extraction (MAE) has been accepted as a potential and powerful alternative to conventional extraction techniques in the extraction of organic compounds from plant materials. The mechanical effects of the internal heating based on conduction and dielectric polarization caused by microwave irradiation, and pressure builds up within the cells of the sample leading to an efficient delivery to materials through molecular interaction with the electromagnetic field and a rapid transfer to energy to the extraction

solvent and raw materials (Eskilsson and Bjorklund, 2000). The MAE process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of ions, which enhance the penetration of solvent into matrix and release the intracellular product by disrupting the cell wall, allowing the dissolution of components to be extracted.

To the best of our knowledge, the investigation of the microwave effects on polysaccharide yield and the corresponding antioxidant activities in *A. blazei Murrill* is rather limited. In the present study, Box-Behnken design (BBD) was used to optimize the process parameters of extraction of crude polysaccharides from the fruiting body of *A. blazei Murrill*. Furthermore, we have compared MAE with the others conventional extraction of the yield, extraction kinetics and the antioxidant properties of polysaccharides obtained using these methods.

Materials and Methods

Mushroom samples Fresh fruiting bodies of *A. blazei Murrill* were obtained from the farm of Zhejiang Academy of Agricultural Science, they were air-dried in an oven at 40°C and ground into a fine powder in a mill before analysis. Voucher specimens were deposited at the Herbarium of Institute of Horticulture, Zhejiang Academy of Agricultural Sciences, Hangzhou, China.

Extraction procedure

Microwave-assisted extraction (MAE) MAE was carried out using microwave experiment equipment (MDS-8, Shanghai SINEO Microwave Chemistry Technology CO., Ltd, Shanghai, China) with adjustable power setting ranging from 200 to 1000 W. It was equipped with ten 100mL closed polytetrafluoroethylene (PTFF) vessels, a power sensor, a temperature sensor and a temperature controller. The powdered *A. blazei Murrill* was transferred into PTFE extraction vessel and the polysaccharides were extracted under different MAE conditions. The ranges of the variables studied are listed in Table 1. After extraction, the vessel was allowed to cool at room temperature, filtered and freeze-dried to obtain crude polysaccharide (PMAE).

Heat reflux extraction (HRE) The powdered mushroom (5.0 g) was dispensed into a round-bottom flask with 100 mL

Table 1. BBD (Box-Behnken design) and the response values for the yields of polysaccharide in *Agaricus blazei Murrill* with the microwave-assisted extraction.

Runs	Time (min)	Microwave power (W)	Temperature (°C)	Water/raw material	Yield [a] (%)
1	−1 (20)	−1 (200)	0 (70)	0 (30)	7.18
2	−1 (20)	1 (400)	0 (70)	0 (30)	11.67
3	1 (40)	−1 (200)	0 (70)	0 (30)	8.06
4	1 (40)	1 (400)	0 (70)	0 (30)	12.36
5	0 (30)	0 (300)	−1 (60)	−1 (20)	8.28
6	0 (30)	0 (300)	−1 (60)	1 (40)	9.89
7	0 (30)	0 (300)	1 (80)	−1 (20)	10.57
8	0 (30)	0 (300)	1 (80)	1 (40)	11.38
9	−1 (20)	0 (300)	0 (70)	−1 (20)	9.08
10	−1 (20)	0 (300)	0 (70)	1 (40)	11.44
11	1 (40)	0 (300)	0 (70)	−1 (20)	9.48
12	1 (40)	0 (300)	0 (70)	1 (40)	10.23
13	0 (30)	−1 (200)	−1 (60)	0 (30)	5.86
14	0 (30)	−1 (200)	1 (80)	0 (30)	8.91
15	0 (30)	1 (400)	−1 (60)	0 (30)	11.61
16	0 (30)	1 (400)	1 (80)	0 (30)	12.80
17	−1 (20)	0 (300)	−1 (60)	0 (30)	10.29
18	−1 (20)	0 (300)	1 (80)	0 (30)	11.09
19	1 (40)	0 (300)	−1 (60)	0 (30)	10.40
20	1 (40)	0 (300)	1 (80)	0 (30)	11.38
21	0 (30)	−1 (200)	0 (70)	−1 (20)	7.36
22	0 (30)	−1 (200)	0 (70)	1 (40)	8.10
23	0 (30)	1 (400)	0 (70)	−1 (20)	11.44
24	0 (30)	1 (400)	0 (70)	1 (40)	11.84
25	0 (30)	0 (300)	0 (70)	0 (30)	11.15
26	0 (30)	0 (300)	0 (70)	0 (30)	11.32
27	0 (30)	0 (300)	0 (70)	0 (30)	11.09

[a] Mean values (n = 3).

of water, the flask was placed into a bath and connected with the condenser, and then allowed to reflux at 90 ± 2°C for three 1 h cycles. After extraction, the extracts were filtered and freeze-dried to obtain crude polysaccharide (PHRE).

Ultrasonic extraction (UE) The powdered mushroom (5.0 g) was put into a conical flask and 100 mL of water was added to it, and the mixture was then extracted in an ultrasonic bath (Kunshan Ultrasonic Instrument Co. Ltd., China) with the power of 100 W at 25°C for three 40 min cycles. After extraction, the extracts were filtered and freeze-dried to obtain crude polysaccharides (PUE).

Maceration extraction (ME) The materials (5.0 g) were accurately weighted, mixed and added to a 250 mL flask with 100 mL of water and macerated at 25°C for three 12 h cycles. After extraction, the extracts were filtered and freeze-dried to obtain crude polysaccharides (PME).

The protein was removed by the Sevage method, combined with papain, according to Zhang *et al.* (2006). Phenol-sulfuric acid method (Dubois *et al.*, 1956) was employed for the measurement of carbohydrate contents of PMAE using glucose as the standard.

Box-Behnken design (BBD) BBD was employed to statistically optimize the parameters and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the yields of polysaccharide. According to the principle of BBD, extraction time, microwave power, extraction temperature and ratio of water to raw material, which were identified to have strong effects on the yields were taken as the variables tested in a 27-run experiment. The four factors chosen for this study were designated as X_1, X_2, X_3 and X_4 and were prescribed into three levels, coded 1, 0, −1 for high, intermediate and low value, respectively. Test variables were coded according to the following equation:

$$X_i = (X_i - X_0) / \Delta X \quad (1)$$

where X_i is the coded value of an independent variable: X_i is the actual value of an independent variable; X_0 is the actual value of an independent variable at centre point; and ΔX is the step change value of an independent variable. All experiments were performed in triplicate and the averages of polysaccharide yield were taken as response. For predicting the optimal point, a second-order polynomial model was fitted to correlate relationship between independent variables and response (polysaccharide yield). For the three factors, the equation was

$$Y = A_0 + \sum_{i=1}^{4} A_i X_i + \sum_{i=1}^{4} A_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} A_{ij} X_i X_j \quad (2)$$

where Y is the response variables (yields of polysaccharides in real values). The percentage of polysaccharides yield was calculated as the polysaccharides content of extraction divided by dried sample weight. A_0, A_i, A_{ii} and A_{ij} are the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are independent variables ($i \neq j$).

Analysis of the experimental design and data were carried out using SAS software (Version 8.0). Analysis of variance (ANOVA) was performed, and the fitness of the polynomial model equation was equation was expressed by the coefficient of determination R^2. Its statistical significance was checked by F-test at a probability of 0.001, 0.01 or 0.05. The significances of the regression coefficients were also tested by F-test.

Assay for antioxidant activities

Hydroxyl radical scavenging assay The hydroxyl radical assay was measured by the method of Ghiselli *et al.* (1998) with a minor modification. Polysaccharides were dissolved in deionized water at the concentration of 0.2−8 mg/mL. The sample solution (0.1 mL) was mixed with 0.6 mL of reaction buffer [20 mM phosphate buffer (pH 7.4), 2.67 mM deoxyribose, and 100 μm ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA)], 0.2 mL of 0.4 mM ferrous ammonium sulfate, 0.05 mL of 2.0 mM Vc, and 0.05 mL of 10 mM H_2O_2. The reaction solution was incubated for 15 min at 37°C and then 1 mL of 1% thiobarbituric acid (TBA) and 1mL of 2% trichloracetic acid (TCA) were added to terminate the reaction. The mixture was boiled for 15 min and cooled at room temperature. The absorbance of the mixture was measured at 532 nm against blank. The capability to scavenge hydroxyl radical was calculated using the following equation:

$$\text{Scavenging activity (\%)} = [1 - A_1 / A_0] \times 100\% \quad (3)$$

where A_0 is the absorbance of mixture solution without sample; A_1 is the absorbance of the test sample mixed with reaction solution.

1,1-diphenyl-2-picryl-hydrazyl(DPPH) radical scavenging assay To evaluate the free radial scavenging activity, all samples were allowed to react with a stable free radical, DPPH (Sanchez-Moreno *et al.*, 1998). The 0.2 mmol/L solution of DPPH in methanol was prepared daily before UV measurement. One milliliter of the polysaccharides of different addition quantities (0.1−1 mg) in water was thoroughly mixed with 2 mL of freshly prepared DPPH and 2 mL of methanol. The mixture was well shaken, allowed to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity, which was analyzed from the graph plotted of inhibition percentage against polysaccharide concentration. Butylated hydroxyanisole (BHA) was used as positive controls. The experiment was carried out in triplicate and averaged. The

DPPH radical scavenging activity was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [A_0 - (A - A_b) / A_0] \times 100\% \quad (4)$$

where A_0 is the absorbance of DPPH solution without sample; A is the absorbance of the test sample mixed with DPPH solution and A_b is the absorbance of the sample without DPPH solution.

Ferrous ion-chelating activity Iron-chelating assay was used for the present investigation of mushroom polysaccharide samples. The chelating of ferrous ions by the extracts and standards was estimated by the method of Dinis *et al.* (1994). 0.2 mL test samples at different concentration (0.2−2 mg/mL) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was determined using the following formula:

$$\text{Chelating activity \%} = [(A_0 - A) / A_0] \times 100\% \quad (5)$$

where A_0 is the absorbance of mixture solution without sample; A is the absorbance of the samples. The control contained $FeCl_2$ and ferrozine, with complex formation molecules. EDTA was used as positive control.

Reducing power The reductive potential of the polysaccharide samples was determined by the method of Oyaizu (1986). The different concentrations of test samples (0.2−2 mg/mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then subjected to centrifugation (10 min, 1000 g). The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reductive potential.

Statistical analysis All tests were conducted in triplicate. Data were reported as mean ± SD. Analysis of variance and significant differences among means were tested by one-way ANOVA using SPSS software (version8.1 for Windows, SPSS Inc., Chicago, IL).

Results and Discussion

Optimization of extraction conditions by BBD Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time (Yin and Dang, 2008). There were a total of 27 runs for optimizing the four individual parameters in the current BBD which was applied to the production of crude PMAE. The values of responses (yield of polysaccharides) at different experimental combination for coded variables are given in Table 1. The percentage yield ranged from 5.86 to 12.80%. By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

$$\begin{aligned} Y = {} & 11.19 + 0.097X_1 + 2.19X_2 + 0.82X_3 + 0.56X_4 - \\ & 0.29X_1^2 - 0.048X_1X_2 + 0.045X_1X_3 - 0.40X_1X_4 - \\ & 0.97X_2^2 - 0.47X_2X_3 - 0.09X_2X_4 - 0.32X_3^2 - \\ & 0.20X_3X_4 - 0.74X_4^2 \end{aligned} \quad (6)$$

where Y is the polysaccharides yield and X_1, X_2, X_3 and X_4 are the coded values for extraction time, microwave power, extraction temperature and water to raw material, respectively.

The ANOVA of the quadratic regression model showed that the value of the determination coefficient ($R^2 = 0.9623$). The value of the adjusted determination coefficient (Adj. $R^2 = 0.9183$) was reasonably close to 1, which indicated a high degree of correlation between the observed and predicted values. At the same time, a low value of coefficient of the variation (CV=4.94%) indicated a high degree of precision and a good deal of reliability of the experiment values.

The p values are used as a tool to check the significance of each coefficient, which in turn may indicate the pattern to the interactions between the variables. The coefficient estimate for the parameter optimization suggested that the independent variables (X_2, X_3, and X_4) and quadratic terms (X_2X_2) significantly affected the polysaccharides yield ($p < 0.05$). The results of the present study showed that the microwave power was the most significant single parameter which influenced polysaccharides yield followed by extraction temperature and ratio of water to raw material.

Optimization of the procedure Eq. (6) allowed the prediction of the effects of the four parameters on the polysaccharides yields. Six independent response surface plots are shown in Fig. 1. Two variables within the experimental range were depicted in one 3D surface plots while the two other variables were kept constant at zero level. The shapes of the contour plots indicated whether the mutual interactions between the variables were significant or not.

Fig. 1A showed that microwave power exhibited a significant effect whereas extraction time showed a weaker effect on the polysaccharides yield. The yield of polysaccharides extracted increased with microwave power. Increased extraction time to a threshold level led to increased polysaccharides yield. Beyond this level, polysaccharides yield slightly decreased. As expected, a greater increase in polysaccharides yield resulted when the extraction temperature was increased.

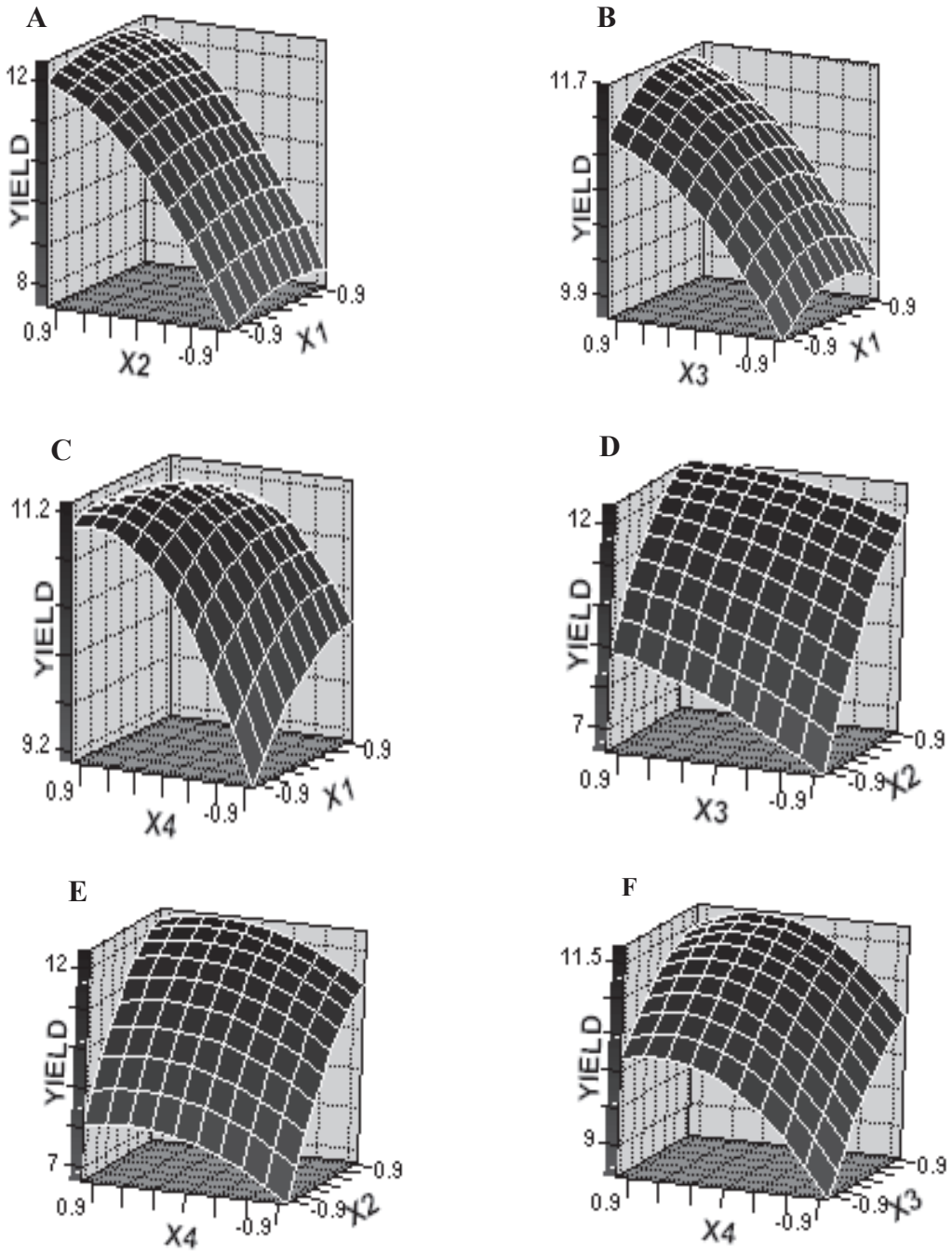

Fig. 1. Response surface plots for the effects of (A) time and microwave power; (B) time and temperature; (C) time and ratio of water to raw material; (D) microwave power and temperature; (E) microwave power and ratio of water to material; and (F) temperature and ratio of water to raw material on the yields of polysaccharide. X_1: extraction time; X_2: microwave power; X_3: extraction temperature; X_4: ratio of water to material.

The temperature curve did not level off at 80°C, which may indicate that a slightly higher temperature is required to achieve maximum increase (Fig. 1B).

As shown in Fig. 1C, while microwave power and extraction temperature were kept at a zero level. Increased extraction time and ratio of water to material up to a respective threshold level led to increased polysaccharides yield and beyond these levels, polysaccharides yield was leveled off. As in the case of polysaccharides extraction, there was a linear increase in the yield of polysaccharides with increase in the extraction temperature and microwave power. So a higher level of extraction temperature and microwave power is required to achieve maximum polysaccharides yield (Fig. 1D).

As Fig. 1E shown, microwave power exhibited significant effect while ratio of water to material showed a weaker effect on the polysaccharides yield. For microwave power, the yield of polysaccharides increased with the microwave power; for ratio of water to material, increased it up to a threshold level led to increased polysaccharides yield. Beyond this level, polysaccharides yield slightly decreased. Fig. 1F showed the extraction yields of polysaccharides increased with increasing of the extraction time (60−80°C) and the ratio of water to material (20−40 ∶ 1).

It could be concluded that the optimal extraction conditions of PMAE were extraction time 29.37 min, microwave power 400 W, extraction temperature 74.64°C and ratio of water to material 32.7 ∶ 1. Among the four extraction parameters studied, microwave power was the most significant factor to affect the yields of polysaccharides, followed by extraction temperature, ratio of water to material and extraction time.

Validation of the models In order to validate the adequacy of the model equations, a verification experiment was carried out under the optimal conditions mentioned above. Under the optimal conditions, the model predicted a maximum response of 12.335%. A mean value of 12.352 ± 0.51% (n = 5), obtained from real experiments, demonstrated the validation of the extraction model. The good correlation between these results confirmed that the model was adequate for reflecting the expected optimization.

Comparison of different extraction techniques In order to compare the MAE techniques with the others conventional methods the extraction efficiency of polysaccharide from *A. blazei Murrill*. The optimal conditions (temperature and time) of HRE, UE and ME were determined using the single-factor method with the same of ratio of water to material 30:1. The extraction yields of polysaccharide obtained by four extraction methods under the optimal conditions are summarized in Table 2. The extraction time of MAE, HRE, UE and ME were 29 min, 80 min, 40 min and 12 h, respectively. The extraction temperature of MAE and HRE were 74 and 90°C, and the extraction temperature of UE and ME were room temperature. The extraction yields of polysaccharides obtained using MAE, HRE, UE and ME were 12.35, 11.44, 11.95 and 11.17%, respectively. The main mechanism for the enhanced yield with MAE was disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of ions, which enhance the penetration of solvent into matrix and release the intracellular product by disrupting the cell wall, allowing the dissolution of components to be extracted (Hemwimon *et al.*, 2007).

Extraction kinetics In the extraction process, the extraction yield depends on both the extraction efficiency and chemical change of the target compound. The extraction kinetics of polysaccharide under four extraction methods is shown in Figs. 2A−D.

In MAE, with an increase of extraction time, the extraction yields of polysaccharide increased in the initial 20 min, and then showed no significant change. Using HRE, the yields of polysaccharide reached its maximum in 60 min and did not significantly change from 60 min to 180 min. In UE, the yields of polysaccharide increased with the increase of extraction time up to 40 min and remain constant. As for ME, it possessed a poor capability for the extraction of polysaccharide was observed with increasing extraction times.

Antioxidant activity analysis The hydroxyl radical generated by Fenton reaction in the system, was scavenged by polysaccharide extracted with different methods. The hydroxyl radical scavenging effects of all samples are shown in Fig. 3A. Among the four samples, PMAE exhibited the strongest scavenging activity. At the concentration of 0.2−8.0 mg/mL, the scavenging effect was 16.5−82.7% for PMAE, 6.3−45.6% for PHRE, 5.7−44.3% for PME, 9.6−49.6% for PUE and 40.3−99.8% for BHA. The IC_{50} value for PMAE was 3.48 mg/mL. However, the hydroxyl radical scavenging efficiency of PHRE, PME and PUE was very low. The above results indicate that polysaccharide extracted with micro-

Table 2. Comparison of microwave-assisted extraction (MAE) and conventional methods under the optimal conditions.

Method	Extraction time	Extraction temperature	Yield [a] (%)
MAE	29 min	74°C	12.35
UE [b]	40 min	room temperature (25°C)	11.95
HRE [b]	80 min	90°C	11.44
ME [b]	12 h	room temperature (25°C)	11.17

[a] Mean values (n = 5).

[b] The optimal conditions (temperature and time) of HRE, UE and ME were determined using the single-factor method with the same of ratio of water to material 30 ∶ 1.

MAE: microwave-assisted extraction; HRE: heat reflux extraction; UE: ultrasonic extraction; ME: maceration extraction.

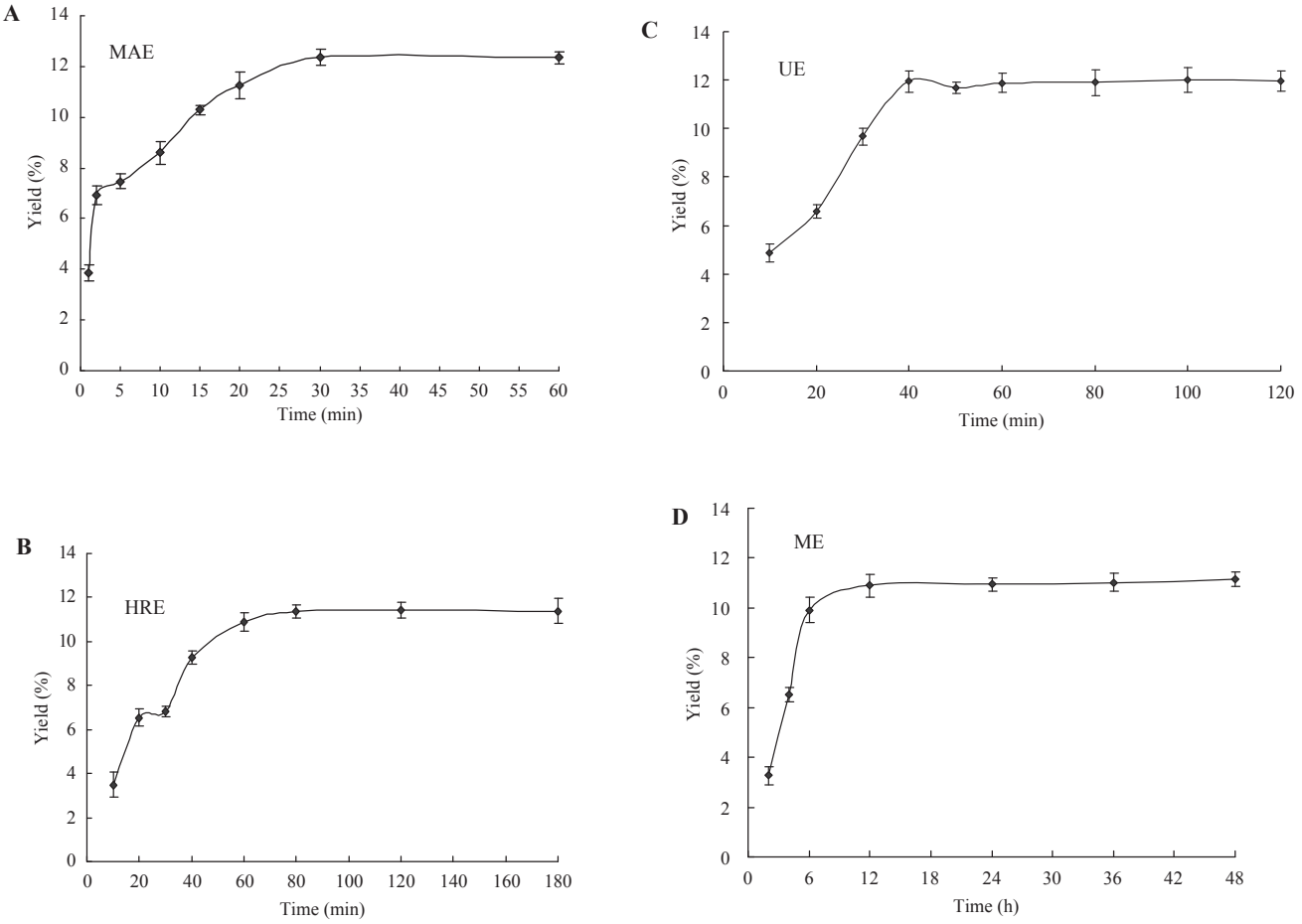

Fig. 2. Extraction kinetics of polysaccharides in *Agaricus blazei murrill* by different extraction techniques. MAE: microwave-assisted extraction; HRE: heat reflux extraction; UE: ultrasonic extraction; ME: maceration extraction. Data are presented as mean ± SD (n = 3).

wave-assisted method has significant scavenging ability on hydroxyl radical.

Fig. 3B described the scavenging ability of polysaccharides obtained with different methods on DPPH radical. In the concentration ranged from 0.1−0.8 mg/mL, the scavenging ability on DPPH radical of PMAE was much higher than those of PHRE, PUE and PME. The higher concentration the higher level of scavenging ability was found for all polysaccharides used in the test. However, the scavenging ability of them was lower than that of BHA. The effect of antioxidants on DPPH radical scavenging was thought to result from their hydrogen donating ability (Baumann *et al.*, 1979). These results revealed the different test polysaccharides can react with free radicals, which are the major initiator of the antioxidative chain of fat, thereby terminating the chain reaction.

As shown in Fig. 3C, the metal chelating activity of tested polysaccharides increased with increasing concentrations used in the test, PUE showed higher metal chelating activity than PME ($p < 0.05$). The IC_{50} of PUE, PMAE and PHRE were 1.48, 1.81 and 1.98 mg/mL. Compared with EDTA, the chelating ability of these polysaccharides on ferrous ion was weaker. Ferrous ions could stimulate lipid peroxidation by Fenton reaction, and also accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Gordon, 1990). Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions (Halliwell, 1991). Our data on the ferrous ions chelating effects of these tested samples suggested that it would be somewhat beneficial to protect against oxidative damage.

In the reducing power assay, the yellow color of test solution changes into various shades of green and blue colors depending on the reducing power of antioxidant samples. Earlier authors have observed a direct correlation between antioxidant activities and reducing power (Siddhuraju and Becker, 2007). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Fig. 3D depicts the reducing power of the polysac-

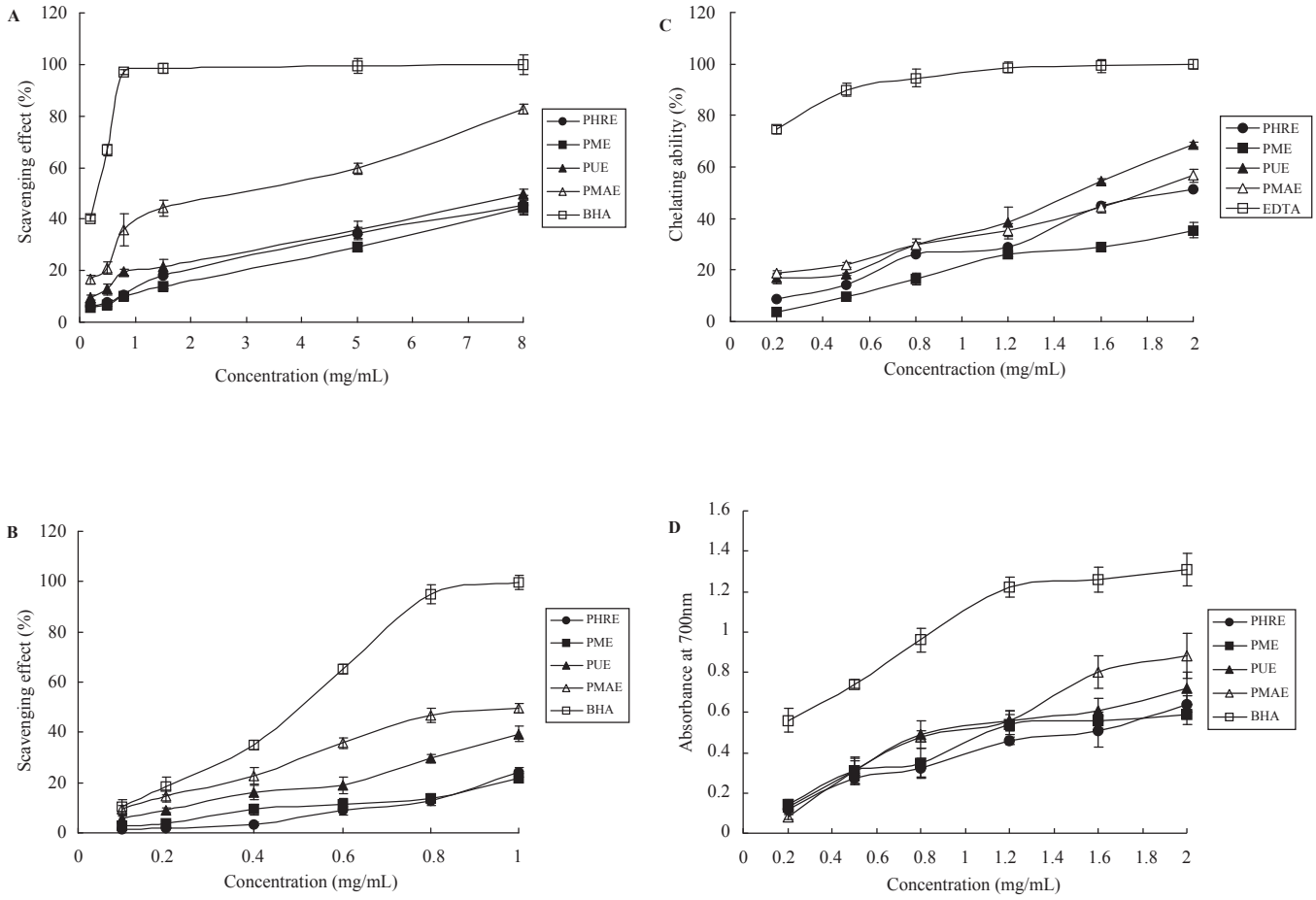

Fig. 3. Antioxidant effect of PMAE, PHRE, PUE and PME with various methods : (A) scavenging activity of hydroxyl radical : (B) scavenging activity of DPPH radicals (C) chelating effect on ferrous ions : (D) reducing power; MAE: Microwave-assisted extraction; HRE: Heat reflux extraction ; UE: Ultrasonic extraction; ME: Maceration extraction. Data are presented as mean ± SD (n = 3).

charides extracted with different methods from *A. blazei Murrill*. The concentration was found to affect the reducing power. The reducing power of the four tested polysaccharides showed similar activity (not significant) and less than that of BHA. The reducing capacity of various examined polysaccharides might be due to its hydrogen-donating ability. Therefore, the examined polysaccharides might contain reductions, which could react with free radicals to stabilize and terminate radical chain reactions.

In all case, the antioxidant activities of PMAE were much higher than those of PUE, PHRE and PME. Earlier authors have revealed that water solubility, molecular weight, molecular structure, polarity and intramolecular hydrogen bonds were the major factors to the antioxidant activity of polysaccharides (Zhang *et al.*, 1998; Ueda *et al.*, 1996; Yanagimoto *et al.*, 2002). Microwave heating results in polarization of polar bonds (such as the C-O-C glycosidic linkages) and increases the molecular reactivity. Therefore, microwave heating might cause a hydrolytic cleavage of polysaccharide chains as well as breaking intermolecular hydrogen bonds (Tao and Xu, 2008). The present study confirmed the results that PMAE, which was degraded during microwave heating processing, showed higher antioxidant activities than polysaccharides extracted by conventional methods. Interesting, we also found that PUE showed higher antioxidant activities than PME and PHRE. Some reports have supported this result that ultrasonic treatment could degrade the polysaccharides to lower the viscosity and increase the solubility (Wang *et al.*, 2010; Liu *et al.*, 2006; Lii *et al.*, 1999).

Conclusions

An efficient process of MAE had been developed for the extraction of polysaccharides from *A. blazei Murrill* with enhanced yield. BBD was used for optimizing extraction parameters in this work. Results showed that extraction time 29.37 min, microwave power 400 W, extraction temperature 74.64°C and ratio of water to material 32.7 : 1 were the best conditions to produce crude PMAE. Under the most suit-

able conditions, maximum yield of polysaccharides can be achieved. Compared to conventional extraction techniques, MAE process required less extraction time and provided higher extraction efficiency. On the basis of the results in this study, it was clearly indicated that PMAE had effective antioxidant activity against various oxidative systems in vitro. The antioxidant mechanisms of PMAE may attribute to strong hydrogen donating ability, metal chelating ability and scavenging free radicals activity. In a word, we conclude that MAE has a great potential for extraction of polysaccharide from *A. blazei Murrill*. The results in this study can be referenced for the extraction of other active compounds from microorganisms and herbal plants and PMAE may be useful as a functional food as well as potential therapeutic agent.

Acknowledgements This research was supported by grants from Innovation Group Project in Edible Mushroom of Zhejiang Province (No.2009R05A50B01). We acknowledge all staff for their valuable assistance in conducting this study.

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