Carbohydrate Polymers 88 (2012) 522–529

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Ultrasonic-assisted extraction and antioxidant activity of polysaccharides recovered from white button mushroom (*Agaricus bisporus*)

Yuting Tian[a,b], Hongliang Zeng[a], Zhenbo Xu[c], Baodong Zheng[a,*], Yuexu Lin[d], Chunji Gan[d], Y. Martin Lo[b,*]

[a] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, PR China
[b] Department of Nutrition and Food Science, University of Maryland, College Park, MD 20742, USA
[c] Department of Microbial Pathogenesis, Dental School, University of Maryland, Baltimore, MD 21201, USA
[d] College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, PR China

ARTICLE INFO

Article history:
Received 28 April 2011
Received in revised form 25 July 2011
Accepted 22 December 2011
Available online 2 January 2012

Keywords:
Agaricus bisporus
Polysaccharide
Extraction
Antioxidant activity

ABSTRACT

Ultrasonic-assisted extraction (UAE) of polysaccharides from white button mushroom (*Agaricus bisporus*) was studied. The four parameters, ultrasonic power, extraction temperature, extraction time, and the ratio of water volume to raw material weight (*W*/*M* ratio), were optimized using the central composite design (CCD) with a quadratic regression model built by using response surface methodology (RSM). The optimal extraction conditions found were: ultrasonic power 230 W, extraction temperature 70 °C, extraction time 62 min, and *W*/*M* ratio 30 ml/g, where the highest *A. bisporus* polysaccharides (ABPS) yield of 6.02% was achieved. From HPGPC analysis, the molecular weight of ABPS was 158 kDa. Chemical and spectroscopic studies illustrated ABPS was only composed of Glc with β-type glycosidic bond. In addition, the antioxidative activity of ABPS was investigated by measuring its scavenging ability on DPPH and hydroxyl in vitro. The results indicated that ABPS has good antioxidant activity.

1. Introduction

Mushrooms have been used as food for centuries because they have rich and balanced nutrients with low lipid content, and have good flavor and texture (Chiang, Yen, & Mau, 2006; Liu, Zhou, Zeng, & Ouyang, 2004). Extensive studies have focused on bioactive compounds derived from various mushrooms (Breene, 1990; Dubost, Ou, & Beelman, 2007; Guo, Savelkoul, Kwakkel, Williams, & Verstegen, 2003; Hartman, 1998; Kasuga, Aoyagi, & Sugahara, 1995). *Agaricus bisporus*, commonly known as the white button mushroom (WBM), is one of the most economically important edible mushrooms. It has high contents of polyphenols, ergothioneine, selenium, and polysaccharides (Mayolo-Deloisa, Trejo-Hernandez, & Rito-Palomares, 2009; Vetter & Lelley, 2004). Recently, Jeong et al. (2010) demonstrated immune modulating and antitumor properties of *A. bisporus*. Studies on the use of *A. bisporus* as a dietary supplement for animal feed has shown it has growth-promoting and tissue antioxidant-protective activity (Dalloul & Lillehoj, 2006; Giannenas et al., 2010).

Polysaccharides extracted from wild plants, animals, and fungi are often identified as biological response modifiers due to their antioxidative, anti-viral and anti-complementary activities (Li, Ding, & Ding, 2007; Sun, Liu, & Kennedy, 2010). Polysaccharides with antioxidant activity have been reported in mushrooms. The most commonly studied groups of mushroom are the Ganodermataceae, *Ganoderma* sp. such as Reishi, *Ganoderma lucidum* (Chen, Shen, & Chen, 2009; Jia et al., 2009; Liu et al., 2010), *G. atrum* (Chen, Xie, Nie, Li, & Wang, 2008), *G. tsugae* (Tseng, Yang, & Mau, 2008), as well as *Lentinula edodes* (Wu & Hansen, 2008; Yu, LiHua, Qian, & Yan, 2009). Other species, such as *Cordyceps militaris* (Dong & Yao, 2008; Yu et al., 2007), *Flammulina velutipes* (Bao, Ochiai, & Ohshima, 2010), *Russula virescens* (Sun, Li, & Liu, 2010) and *Lentinus* sp. (Thetsrimuang, Khammuang, & Sarnthima, 2011) have also been reported to contain antioxidative polysaccharides. Commercially available pharmaceutical mushroom polysaccharides come mainly from *L. edodes* and *G. lucidum*. However, little is known in respect with the extraction of polysaccharides from *A. bisporus*.

Hot-water extraction has been widely used to extract plant polysaccharides (Lai & Yang, 2007; Zhu et al., 2009). However, the requirement for high temperatures and extended extraction times has disadvantages (Wang, Cheng, Mao, Fan, & Wu, 2009). Development of an economical and efficient extraction technique for mushroom polysaccharides is of an urgent necessity. Various recently developed novel techniques for the extraction of bioactive substances from plants, include supercritical fluid extraction (Turner, King, & Mathiasson, 2001), microwave-assisted extraction (Wang et al., 2010) and ultrasonic-assisted extraction (Lai, Wen, Li, Wu, & Li, 2010; Ying, Han, & Li, 2011). Compared with the

* Corresponding author. Tel.: +86 591 83739118; fax: +86 591 83739118.
E-mail address: zbdfst@hotmail.com (B. Zheng).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.12.042

first two methods, ultrasonic-assisted extraction has the advantage of accelerating the extraction process, causing less damage to the structural and molecular properties of plant materials (Vilkhu, Mawson, Simons, & Bates, 2008), and can be done at low temperatures (Xu, Zhang, & Hu, 2000). For these reasons ultrasonic methods for assisting the extraction of polysaccharides from plant material are widely used today (Chen et al., 2010; Hromadkova, Ebringerova, & Valachovic, 2002; Pan et al., 2010).

In this work, the extraction variables (ultrasonic power, extraction temperature, extraction time and *W*/*M* ratio) were optimized by employing response surface methodology (RSM) for maximum polysaccharides yield. The chemical composition and preliminary structural characterization of the polysaccharides were studied. The polysaccharides were further investigated for their antioxidant activities by DPPH radical assay and studying their hydroxyl radicals.

2. Materials and methods

2.1. Dried A. bisporus preparation

A. bisporus was purchased in a local commercial market in the mushroom producing area of Fuzhou, Fujian Province, China. All samples were processed as follows: rinse for 15 min under running tap water and oven dry for ca. three days of oven-dry at 70–80 °C, grind with a mill (FW-80, Taisite Co., Tianjin, China) and seal in air-tight plastic bags stored under dry and dark conditions until used.

2.2. Extraction of polysaccharides

The ultrasonic-assisted extraction of polysaccharides from dried *A. bisporus* was performed using an ultrasonic clearer (KQ-400KDV, Kunshan, Zhejiang, China) with thermostatic temperature control. Five grams of dried *A. bisporus* powders were extracted with distilled water in a 250-ml beaker held in the ultrasonic clearer and extracted experimentally at a variety of selected ultrasonic powers at different temperatures, and for different lengths of time. After filtration to remove debris fragments, the filtrate was concentrated using a speed vacuum concentrator (BUCHI 409, Buchi Corp., New Castle, DE, USA). The protein was removed as described previously (Matthaei, Jones, Martin, & Nirenberg, 1962), and the solution was then precipitated with a threefold of volume of 95% (v/v) ethanol overnight at 4 °C. The resulting precipitate was collected by centrifugation at 4 °C at 4500 rpm for 20 min (PM180R, ALC International, Milan, Italy), washed with distilled water, centrifuged, then dried by freeze-drying (MCFD5505, SIM, Newark, DE, USA). The ABPS were weighted with a balance (AUY 220, Shimadzu, Japan). The percentage polysaccharides yield (%) is calculated as follows:

$$\text{Yield}\ \ (\%,\ \text{w/w}) = \frac{\text{Weight of dried crude extraction}}{\text{Weight of } A.\ bisporus\ \ \text{powder}} \times 100 \qquad (1)$$

2.3. Optimization of ultrasonic-assisted treatment

The optimal conditions for ultrasonic-assisted extraction were determined by response surface methodology (RSM), with the standard of five-levels, four-factors central composite design (CCD). Table 1 shows the range and center point values of the four independent variables based on the results of the above experiments. The CCD in the experimental design consisted of 30 treatments including 2^4 factorial points, and eight axial points ($\alpha = 2$) and six replicates of the central point. The ultrasonic power (W, X_1), extraction temperature (°C, X_2), extraction time (min, X_3), and *W*/*M* ratio (ml/g, X_4) were chosen as the independent variables, and the extraction yield of polysaccharides (%, *Y*) was chosen as the response (Table 2). Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

2.4. Control experiment

The traditional hot water extraction (HWE) was carried out in a water bath (HH-6 Guohua Wiring Company, Shanghai, China) at the optimal extraction condition: extraction temperature of 95 °C, extraction time of 125 min, and *W*/*M* ratio of 30:1 based on the preliminary three-factor and three-level designed orthogonal optimal experiment.

Microwave-assisted extraction (MAE) was carried out in a ME1-3 L microwave extraction apparatus (Wuxi Pulaima Instrument Co., Ltd., Jiangsu, China) with a power of 360 W, extraction temperature of 75 °C, extraction time of 30 min, and *W*/*M* ratio of 30:1 based on the preliminary optimal experiment.

2.5. Isolation and purification of ABPS

DEAE-cellulose-52 column (1.6 cm × 30 cm) was applied to purify the ABPS. The column was first eluted with distilled water followed by 0.3 M and the 0.6 M NaCl. The only main peak was further fractionated on a Sephadex G-100 column (1.6 cm × 30 cm) eluted with 0.1 M NaCl to yield single fraction. The total carbohydrate was determined by phenol–sulfuric acid colorimetric method.

2.6. Analysis of carbohydrate composition

The molecular weight of ABPS was determined by gel-permeation chromatography (GPC), in combination with a high-performance liquid chromatography (HPLC) instrument (LC-2010A, Shimadzu, Tokyo, Japan) equipped with an Ultrahydrogel linear column (10 μm, 7.8 mm × 300 mm, Waters, USA) and a refractive index (RI) detector (RID-10A, Shimadzu, Tokyo, Japan). The column was eluted with 0.05 mol/l NaCl at a flow rate of 0.8 ml/min. The HPLC system was precalibrated with T-series Dextran standards (T-10, T-40, T-70, and T-500).

The ABPS sample (2 mg) was hydrolyzed in 2 ml of 2 M trifluoroacetic acid (TFA) at 100 °C for 8 h. The chemical composition of ABPS was measured by a HPLC system (LC-2010A, Shimadzu, Tokyo, Japan) with a refractive index (RI) detector and ZORBAX Carbohydrate Analysis Columns (5 μm, 4.6 mm × 250 mm, Agilent, USA) at 25 °C. The column was eluted with acetonitrile and water (85:15, V/V) at a flow rate of 1.0 ml/min.

2.7. FT-IR and NMR spectroscopy

Infrared spectra of ABPS were measured with a FT-IR spectrophotometer (Thermo Nicolet, AVATAR360, USA) equipped with EZ Omnic 6.1a software. The sample was ground with KBr powder and then pressed into 1 mm pellets for FTIR measurement in the frequency range of 4000–400 cm^{-1}. Thirty-two scans were run in each spectrum at a resolution of 4 cm^{-1}.

^1H NMR and ^{13}C NMR spectra were obtained on NMR spectrometer (AVANCE III 400, Bruker, Germany) at 25 °C. Polysaccharides sample (30 mg) was dissolved in D_2O and lyophilized three times. The deuterium-exchanged ABPS, dissolved in D_2O again, were detected on NMR spectrometer using tetra-methyl silane (TMS) as an internal standard.

Table 1
The levels of variables employed in the present study for the construction of Central Composition Design (CCD).

Variables	Levels				
	2	−1	0	1	2
Ultrasonic power X_1, W	160	200	240	280	320
Extraction temperature X_2, °C	60	65	70	75	80
Extraction time X_3, min	20	40	60	80	100
W/M ratio X_4, ml/g	20	25	30	35	40

2.8. Antioxidant activity of polysaccharides

2.8.1. Assay of DPPH radical scavenging activity

The free radical scavenging activity of ABPS was determined by using a modified protocol based on (Yang, Zhao, Shi, Yang, & Jiang, 2008). Briefly, different volumes of the ABPS solution (1 mg/ml) were added to 2 ml DPPH solution (800 mM in dehydrated alcohol) and the final reaction volume was made up to 4 ml with 70% ethanol. After shaking vigorously, the mixture was incubated at room temperature in the dark for 20 min. The absorbance was measured at 517 nm against a blank (distilled water instead of the ABPS solution). The same procedure was repeated with the synthetic antioxidant, butylated hydroxytoluene (BHT), as a positive control. Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. The capability to scavenge the DPPH radical was calculated by the following equation:

$$\text{Scavenging ability}\ (\%) = \frac{A0 - A1}{A0} \times 100\% \quad (2)$$

A_0 and A_1 are the absorbance of control (without sample) and sample, respectively.

2.8.2. Assay of hydroxyl radicals scavenging activity

The hydroxyl radicals scavenging activity of ABPS was measured using a previous method with modification (Sun, Li, et al., 2010). In brief, the reaction mixture contained 1 ml of brilliant green (0.435 mM), 1.0 ml of EDTA–ferrous ion solution (9 mM), 1.0 ml of H_2O_2 (8.8 mM) and different volumes of the ABPS solution (1 mg/ml). The final reaction volume was made up to 4 ml with distilled water. After incubation at room temperature for 20 min, the absorbance of the mixture was measured at 624 nm against a blank (distilled water instead of the ABPS solution). The same procedure was repeated with vitamin C (Vc), as a positive control. The hydroxyl radical-scavenging activity was expressed as following:

$$\text{Scavenging ability}\ (\%) = \frac{A0 - A1}{A0} \times 100\% \quad (3)$$

A_0 and A_1 are the absorbance of control (without sample) and sample, respectively.

2.9. Statistical analysis

Means ± SD had been used in the implementation of results and statistical analysis was performed using the software Design-Expert® 7.0.0 (Stat-Ease Inc., Minneapolis, MN, USA). Data were then analyzed by analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range tests. All analyses were performed in triplicate.

Table 2
Experimental design and result of factors chosen for the trials of CCD.

No.	x_1	x_2	x_3	x_4	Yield of polysaccharide (%)	
					Experimental	Predicted
1	−1	−1	−1	−1	4.43	4.25
2	1	−1	−1	−1	3.58	3.67
3	−1	1	−1	−1	3.87	4.03
4	1	1	−1	−1	4.17	4.12
5	−1	−1	1	−1	4.27	4.37
6	1	−1	1	−1	3.72	3.54
7	−1	1	1	−1	4.75	4.54
8	1	1	1	−1	4.25	4.39
9	−1	−1	−1	1	4.50	4.58
10	1	−1	−1	1	3.79	3.83
11	−1	1	−1	1	3.86	3.87
12	1	1	−1	1	3.68	3.80
13	−1	−1	1	1	4.81	4.69
14	1	−1	1	1	3.63	3.70
15	−1	1	1	1	4.26	4.39
16	1	1	1	1	4.06	4.07
17	−2	0	0	0	4.36	4.40
18	2	0	0	0	3.60	3.50
19	0	−2	0	0	4.50	4.57
20	0	2	0	0	4.85	4.72
21	0	0	−2	0	3.91	3.80
22	0	0	2	0	4.13	4.18
23	0	0	0	−2	3.80	3.87
24	0	0	0	2	4.00	3.87
25	0	0	0	0	5.96	5.88
26	0	0	0	0	5.92	5.88
27	0	0	0	0	5.96	5.88
28	0	0	0	0	5.75	5.88
29	0	0	0	0	5.87	5.88
30	0	0	0	0	5.83	5.88

3. Results and discussion

3.1. Fitting to model

Four variables, including X_1 (ultrasonic power), X_2 (extraction temperature), X_3 (extraction time) and X_4 (W/M ratio) were selected and separately optimized using CCD design. Table 2 shows the complete design matrix together with the response values obtained. The yield of ABPS ranged from 3.58 to 5.96%, and reached maximum with the W/M ratio of 30 ml/g at 240 W, 70 °C, and a 60 min treatment time. Trials No. 24-30 in Table 2 were used to determine the experimental error. By applying multiple regression analysis on the experimental data, the response and test variables were found to correlate by the following second-order polynomial equation:

$$\begin{aligned} Y = {} & 5.85 - 0.22x_1 - 0.036x_2 + 0.096x_3 - 0.0021x_4 \\ & + 0.17x_1x_2 - 0.0062x_1x_3 - 0.0042x_1x_4 + 0.10x_2x_3 \\ & - 0.12x_2x_4 - 0.00062x_3x_4 - 0.48x_1x_1 - 0.31x_2x_2 \\ & - 0.47x_3x_3 - 0.50x_4x_4 \end{aligned} \quad (4)$$

where Y is the yield of ABPS (%), and x_1, x_2, x_3, and x_4 are the coded variables for ultrasonic power, extraction temperature, extraction time and W/M ratio, respectively.

Table 3 summarized the results of the analysis of variance, goodness-of-fit and the adequacy of the mode. The R-value for Eq. (4) was 0.9910, which was relatively high (close to unity), indicating a close agreement between experimental and predicted values of the ABPS yield. The R^2_{adj} is the correlation measure for testing the goodness-of-fit of the regression equation. The higher it is the better degree of correlation between the actual and predicted values (Zhong & Wang, 2010). The value R^2_{adj} for Eq. (4) was 0.9652, which indicates that 96.52% of the total variation in the yield was attributed to the experimental variables studied.

The adequacy of the model was further justified through analysis of variance (ANOVA). Table 3 lists the ANOVA quadratic model for the yield of ABPS. From the analysis, the F-value of 252.08 and P-value < 0.0001 indicates the response surface quadratic model was significant. The significance of each coefficient was tested using the P-value in Table 4. The corresponding variables become more significant as the F-value becomes larger and the P-value becomes smaller. Also, the P-value can be used to check the interaction strength between each independent variable (Muralidhar, Chirumamilla, Ramachandran, Marchant, & Nigam, 2001). In this case, the independent variables (x_1 and x_3), the interaction terms (x_1x_2, x_2x_3, and x_2x_4), and all two quadratic terms (x_1^2, x_2^2, x_3^2, and x_4^2) significantly affected the yield of ABPS.

3.2. Analysis of response surface

Based on the above data (Eq. (4)) using RSM, tri-dimensional response surface plots and two-dimensional contour plots were constructed to visualize the relationship between responses and the levels of the processing variables and the interactions between two variables. The tri-dimensional surface plots were obtained by plotting the response on the Z-axis against any two variables while keeping other variables at their optimal level.

Figs. 1a and 2a a show the effects of ultrasonic power (X_1) and extraction temperature (X_2) are shown in. The yield of ABPS initially increases with an increase in ultrasonic power and extraction temperature. With the treatment time set at 62.4 min and the W/M ratio set at 30 ml/g, the highest ABSP yield should be found with an ultrasonic power and extraction temperature range of 203.3–243.9 W and 65.5–72.6 °C, respectively.

Figs. 1b and 2b shows effects of ultrasonic power (X_1) and extraction time (X_2) on the yield of ABPS. With the extraction temperature set at 70.1 °C and the W/M ratio at 30.0 ml/g, the optimal ABPS yield should be found with an ultrasonic power range of 222.2–244.8 W with extraction time between 52.7 and 74.0 min.

Figs. 1c and 2c show the effects of ultrasonic power (X_1) and W/M ratio (X_4) on the yield of ABPS. With the extraction temperature and extraction time set at 70.1 °C and 62.4 min, respectively, the optimal ABPS yield should be found with an ultrasonic power of 210.9–253.8 W and the W/M ratio of 27.5–32.9 ml/g.

Figs. 1d and 2d show the effects of extraction temperature (X_2) and extraction time (X_3) on the yield of ABPS. With the ultrasonic power set at 230.5 W and the W/M ratio at 30.0 ml/g, the highest ABPS should be found with an extraction temperature of 66.4–71.9 °C and an extraction time of 55.4–64.8 min.

Figs. 1e and 2e show the effects of extraction temperature (X_2) and W/M ratio (X_4) on the yield of ABPS. With an ultrasonic power at 230.5 W and an extraction time of 62.4 min, the optimal ABPS yield should be found with the extraction temperature of 67.7–74.4 °C and a W/M ratio of 30.0–31.3 ml/g.

Figs. 1f and 2f show the effects of extraction time (X_3) and W/M ratio (X_4) on the yield of ABPS are shown in. With the ultrasonic power at 230.5 W and an extraction temperature of 70.1 °C, the highest ABPS yield should be found with an extraction time of 56.1–68.8 min and a W/M ratio of 27.4–32.7 ml/g.

3.3. Experimental validation of the optimized condition

The optimal values of the four studied variables obtained from the model using the Design-Expert software were an ultrasonic power, an extraction temperature, an extraction time and a W/M ratio of 230.5 W, 70.1 °C, 62.4 min, and 30.0 ml/g, respectively. The model predicted a maximum ABPS yield of 5.91% under the optimal conditions. The optimal conditions were determined using the simplex method and the maximum desirability for the extraction yield of ABPS to verify the predictive capacity of the model. A mean value of 6.02 ± 0.07% (N = 5) was obtained from laboratory experiments. This proves the response model constructed was adequate in predicting optimal conditions achievable in laboratory settings.

3.4. Comparison of UAE, HWE, and MAE

In the control experiment, the yield of ABPS obtained by HWE and MAE were 2.36 ± 0.05 and 4.71 ± 0.11%, respectively. Clearly, the application of UAE positively affected the ABPS yield. Under the optimal conditions of UAE, the ABPS yield increased by 155.08% by 27.81% when compared to HWE and MEA, respectively. When compared with HWE, UAE gave higher yields of ABPS with a shorter extraction time and at lower temperatures. In addition, compared with MAE, UAE had a larger ABPS yield and used less power and a lower extraction temperature. Comparisons of the structure and bioactivities between and among these three methods need to be studied further to promote the commercial application of UAE for use in the field of bioactive compounds.

3.5. Chemical composition and preliminary structural characterization of ABPS

HPGPC has often been employed to determine the molecular weight of polysaccharides. The molecular weight of ABPS was estimated to be 158 kDa. The HPGPC profile also demonstrated ABPS had a single and symmetrically sharp peak indicating ABPS is a homogeneous polysaccharide. HPLC analysis showed ABPS was only composed of one kind of monosaccharides: D-glucose.

IR spectra of ABPS showed absorption bands at 3391, 2931, 2361, 1624, 1406, 1080, 936, and 881 cm^{-1} (Fig. 3). A broad band

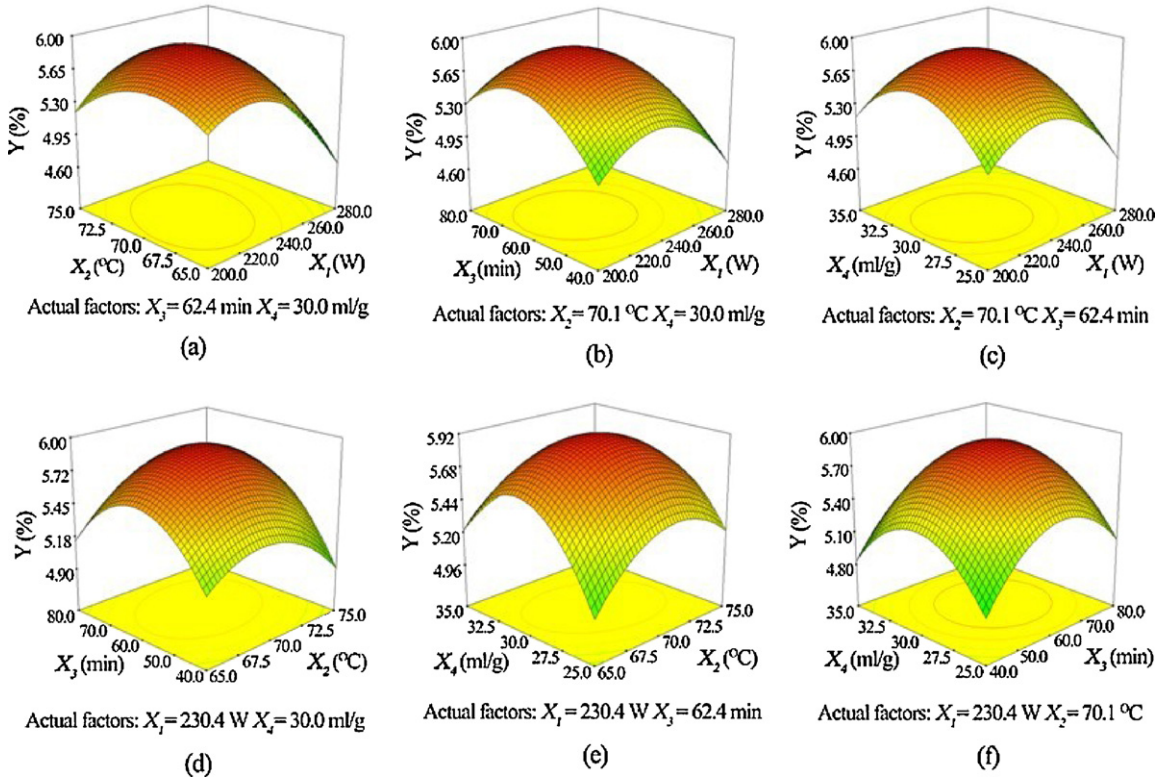

Fig. 1. Tri-dimensional response surface contour plots showing the experimental factors and their mutual interactions on ABPS extraction: (a) $Y=f(X_1, X_2, 42.4, 35.3)$; (b) $Y=f(X_1, 70.1, X_3, 30.0)$; (c) $Y=f(X_1, 70.1, 62.4, X_4)$; (d) $Y=f(230.4, X_2, X_3, 30.0)$; (e) $Y=f(230.4, X_2, 62.4, X_4)$; (f) $Y=f(230.4, 70.1, X_3, X_4)$. Y, yield for ABPS (%); X_1, ultrasonic power (W); X_2, extraction temperature (°C); X_3, extraction time (min); X_4, W/M ratio (ml/g).

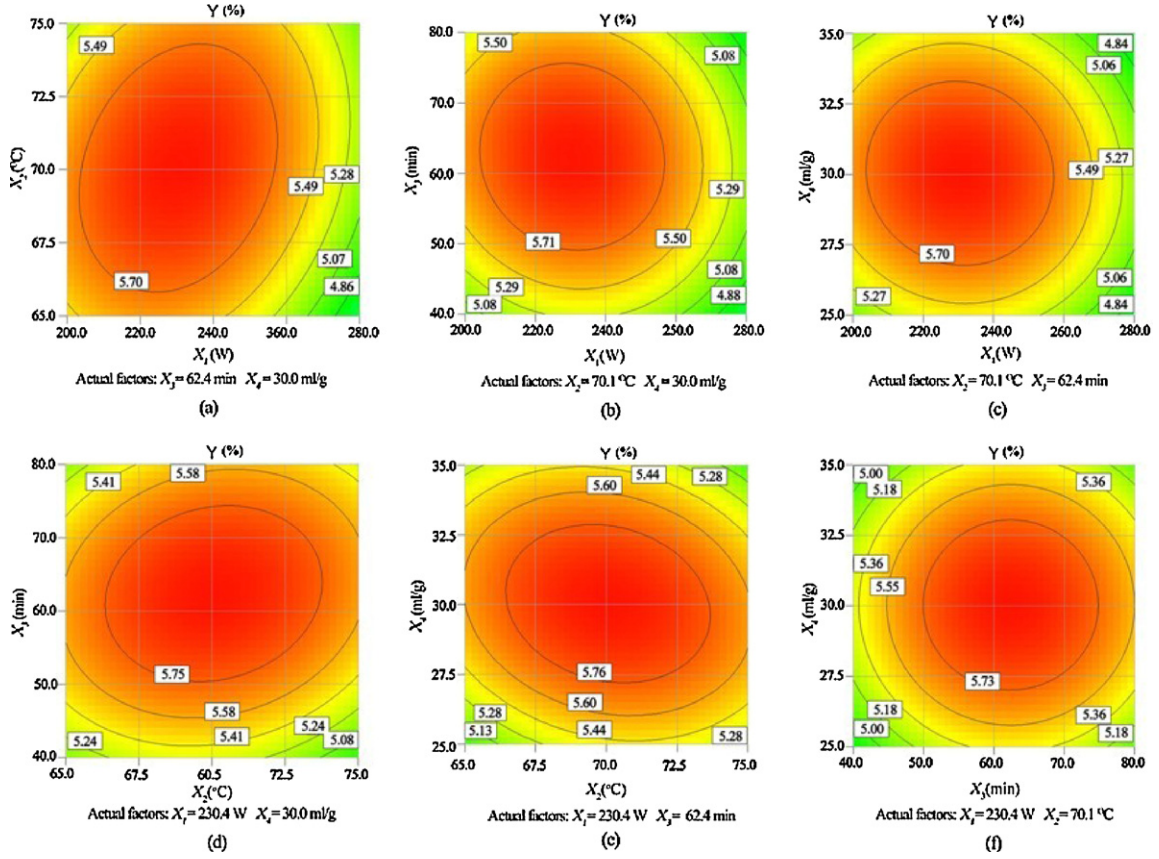

Fig. 2. Two-dimensional contour plots showing the experimental factors and their mutual interactions on ABPS extraction: (a) $Y=f(X_1, X_2, 42.4, 35.3)$; (b) $Y=f(X_1, 70.1, X_3, 30.0)$; (c) $Y=f(X_1, 70.1, 62.4, X_4)$; (d) $Y=f(230.4, X_2, X_3, 30.0)$; (e) $Y=f(230.4, X_2, 62.4, X_4)$; (f) $Y=f(230.4, 70.1, X_3, X_4)$. Y, yield for ABPS (%); X_1, ultrasonic power (W); X_2, extraction temperature (°C); X_3, extraction time (min); X_4, W/M ratio (ml/g).

Table 3
Analysis of variance (ANOVA) testing the fitness of the regression equation.

Source	Sum of squares	df	Mean square	*F*-value	*P*-value
Model	18.13756	14	1.29554	58.47913	<0.0001
Residual	0.332308	15	0.022154		
Lack of fit	0.298425	10	0.029843	4.403714	0.0577
Pure error	0.033883	5	0.006777		
Cor total	18.46987	29			

$R = 0.9910$, $R^2 = 0.9820$, $R^2_{adj} = 0.9652$.

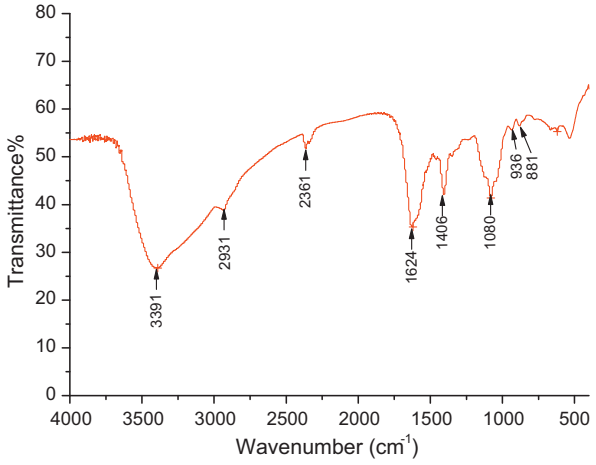

Fig. 3. FT-IR spectroscopy of ABPS.

centered at 3391 cm^{-1} assigned to hydrogen-bonded hydroxyl groups (Wang, Zhang, Di, Liu, & Wu, 2011). The band at around 2931 cm^{-1} was a C—H stretching vibration (Zhang, Ye, & Wang, 2010). The peak at 2361 cm^{-1} was a C—H transiting angle. The absorptions at 1624 cm^{-1} were assigned to the stretching vibrations of the CHO and C=O bonds. The broad absorption bands with strong intensities at 1406 cm^{-1} could be assigned to deforming vibrations of C—H bond (Wang, 2011). There was only one peak (1100 and 1010 cm^{-1}) of ABPS, which indicated the existence of pyranose (Ying et al., 2011). The weak absorption bands at 936 and 881 cm^{-1} were related to the C—H deforming vibrations in β-pyran ring. These results indicated that ABPS possesses typical absorption peak of polysaccharides.

NMR spectroscopy, an important non-destructive tool, was used to investigate the fine structure of polysaccharides, including identification of monosaccharide composition, elucidation of α- or β-anomeric configurations, establishment of linkage patterns and sequences of the sugar units in the polysaccharides (Li, Fan, & Ding, 2011). Fig. 4 shows all the NMR chemical shifts of ABPS. In the ^{1}H NMR spectra of ABPS, chemical shifts from 3.520 to 3.752 ppm were designated as the protons of carbons C-2 to C-6 on glycosidic ring (Wu, Sun, & Pan, 2006). Based on the result of monosaccharide analysis, the signal in the ^{1}H NMR of ABPS at δ 3.643, δ 3.520, δ 3.563 and δ 3.427 were attributable to the C-5, C-4, C-3, and C-2, individually (Ma, Qiao, & Xiang, 2011) and the peak at δ 63.16 ppm in the ^{13}C spectra of ABPS could be assigned to C-6 of β-D-glucose (Hromadkova et al., 2002). Of the two types of anomeric protons, signals in the ^{1}H NMR spectra derived from β-anomeric appear in less than 5 ppm (Cui, 2005). Combined with the results of IR data, the signals in the NMR spectra indicated ABPS had β-glycopyranosidic linkages. Further research should be focused on the detailed linkage by methylation analysis.

3.6. Antioxidant activity of ABPS

3.6.1. DPPH scavenging activity of ABPS

DPPH, a stable free radical discovered by Goldschmidt and Renn (Ionita, 2005), has been commonly used as a tool for determining the free-radical scavenging ability of the polysaccharides (Lai et al., 2010; Qiao et al., 2009; Xu et al., 2009). The change of concentration of ABPS was monitored to evaluate the antioxidant ability of ABPS through the DPPH scavenging activity test. Fig. 5 presents the results. BHT was used as a positive control as previously reported by Xie et al. (2010). As shown in Fig. 5, ABPS exhibited a concentration-dependent antiradical activity by inhibiting DPPH free radicals. The DPPH scavenging effect increased by increasing the concentration of ABPS up to 250 μg/ml. Compared with BHT, ABPS showed a higher degree of free radical-scavenging activity under the same conditions. At 250 μg/ml concentration, ABPS was observed to possess significantly ($P < 0.01$) higher (86.1%) free radical-scavenging activity when compared to BHT (83%), suggesting that ABPS has stronger DPPH radical-scavenging activity.

3.6.2. Hydroxyl radical scavenging activity of ABPS

Hydroxyl radicals are the most reactive among reactive oxygen species (ROS) and can be generated in biological cells through

Table 4
Testing of the significance of the regression coefficients associated with different experimental factors.

Factor	Coefficient estimate	df	Standard error	95% CI low	95% CI high	*F*-value	*P*-value
Intercept	5.881667	1	0.060764	5.75215	6.011183	–	–
x_1	−0.22458	1	0.030382	−0.28934	−0.15983	54.64071	<0.0001
x_2	0.03625	1	0.030382	−0.02851	0.101008	1.423565	0.2513
x_3	0.09625	1	0.030382	0.031492	0.161008	10.03605	0.0064
x_4	−0.00208	1	0.030382	−0.06684	0.062675	0.004702	0.9462
x_1x_2	0.169375	1	0.03721	0.090063	0.248687	20.71899	0.0004
x_1x_3	−0.06187	1	0.03721	−0.14119	0.017437	2.765034	0.1171
x_1x_4	−0.04187	1	0.03721	−0.12119	0.037437	1.266426	0.2781
x_2x_3	0.100625	1	0.03721	0.021313	0.179937	7.312768	0.0163
x_2x_4	−0.11938	1	0.03721	−0.19869	−0.04006	10.29193	0.0059
x_3x_4	−0.00062	1	0.03721	−0.07994	0.078687	0.000282	0.9868
x_1x_1	−0.4824	1	0.02842	−0.54297	−0.42182	288.1113	<0.0001
x_2x_2	−0.30865	1	0.02842	−0.36922	−0.24807	117.9435	<0.0001
x_3x_3	−0.4724	1	0.02842	−0.53297	−0.41182	276.2901	<0.0001
x_4x_4	−0.5024	1	0.02842	−0.56297	−0.44182	312.4966	<0.0001

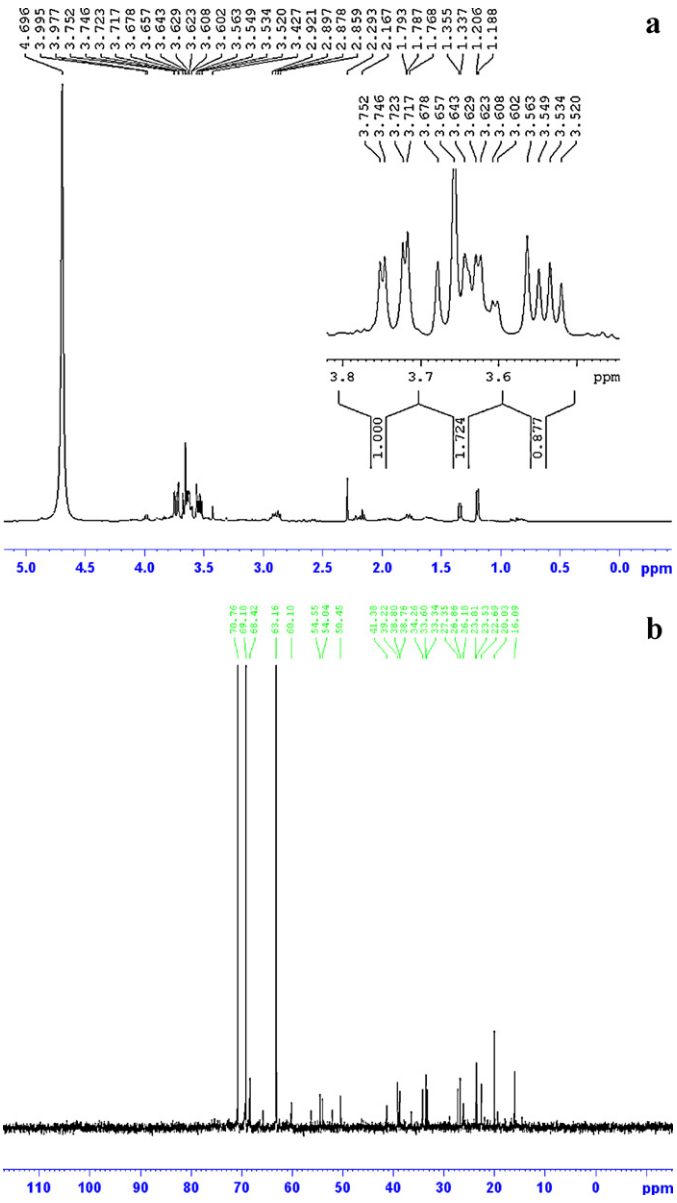

Fig. 4. ^{1}H NMR (a) and ^{13}C NMR (b) spectra of ABPS.

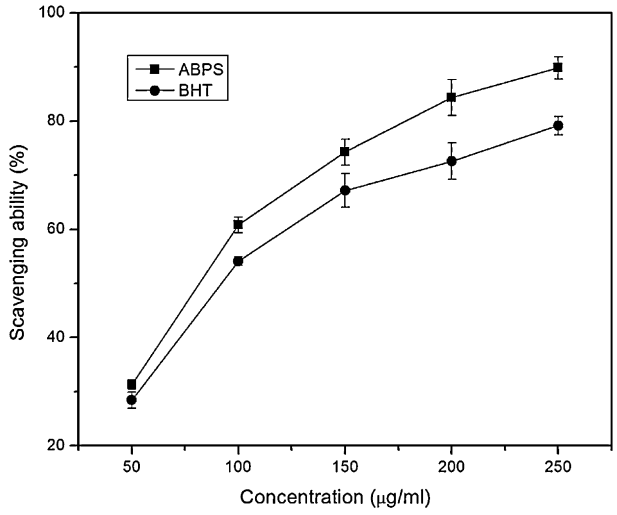

Fig. 5. Free radical-scavenging activity of ABPS and BTH at different concentrations. Data are shown as mean ± SD (n = 3).

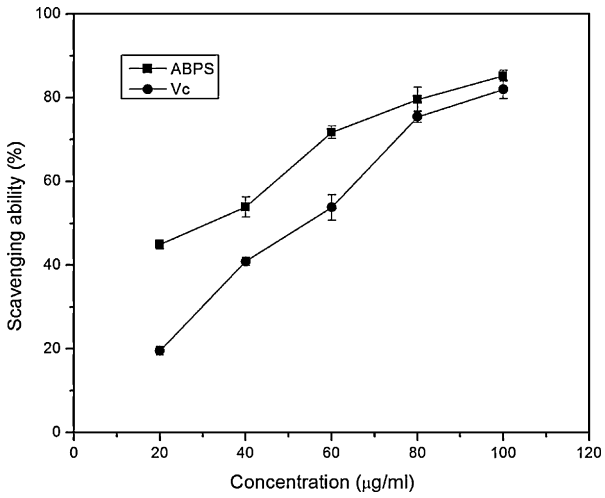

Fig. 6. Hydroxyl radical-scavenging activity of ABPS and Vc at different concentrations. Data are shown as mean ± SD (n = 3).

the Fenton reaction (Yuan et al., 2010). Moreover, hydroxyl radicals can also induce significant damage to adjacent biomolecules among the oxygen radicals (Bin, 2010). So removing hydroxyl radicals is important for antioxidant defense in living cell systems. Fig. 6 shows an analysis of the hydroxyl radical-scavenging activities of ABPS with Vc as positive control (Luo et al., 2010). This shows ABPS exhibited scavenging activity towards hydroxyl radicals in a concentration-dependent manner and the scavenging effect increased based on the concentration of ABPS. The antioxidant activity of ABPS was detected to be higher than that of Vc at each concentration point. When the concentration was below 80 μg/ml, ABPS was observed to possess obviously higher free radical-scavenging activity than that of Vc, suggesting ABPS has a noticeable effect on its hydroxyl radical scavenging activity.

4. Conclusion

Ultrasonic technology was found an effective method for ABPS extraction, as evidenced by the improved yield reached by the optimal extraction conditions identified by response surface methodology (RSM). The highest ABPS was found to be 6.02% (w/w) with 230 watts of ultrasonic power at 70 °C for 62 min with the W/M ratio at 30 ml/g. The experimental values identified under these conditions were closely correlated to the predicted values. Compared with HTW and MAE, UAE was the best method of extracting ABPS. One fraction of polysaccharides was obtained through purification of ABPS by using DEAE-Cellulose-52 column and Sepharose G-100 column chromatography. The molecular weight of ABPS was estimated to be 158 kDa and it consisted of only D-glucose. In addition, the free-radical scavenging capacity of the ABPS extracted in the present study was analyzed and discovered.

Acknowledgements

The authors wish to thank Dr. Chunfeng Yan (Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences) for her assistance in the structural characterization of polysaccharides. The research was financial supported by the China State "11th Five-Year Plan" Scientific and Technological Support Scheme (2007BAD07B05), Special Major Science and Technology in Fujian Province (2008N0007) and the Natural Science Fund in Fujian Province (2006J0190, 2011J05123).

References

Bao, H. N. D., Ochiai, Y., & Ohshima, T. (2010). Antioxidative activities of hydrophilic extracts prepared from the fruiting body and spent culture medium of *Flammulina velutipes*. *Bioresource Technology*, *101*(15), 6248–6255.

Bin, C. (2010). Optimization of extraction of *Tremella fuciformis* polysaccharides and its antioxidant and antitumour activities in vitro. *Carbohydrate Polymers*, *81*(2), 420–424.

Breene, W. M. (1990). Nutritional and medicinal value of specialty mushrooms. *Journal of Food Protection*, *53*, 883–894.

Chen, X. P., Wang, W. X., Li, S. B., Xue, J. L., Fan, L. J., Sheng, Z. J., et al. (2010). Optimization of ultrasound-assisted extraction of Lingzhi polysaccharides using response surface methodology and its inhibitory effect on cervical cancer cells. *Carbohydrate Polymers*, *80*(3), 944–948.

Chen, Y., Xie, M.-Y., Nie, S.-P., Li, C., & Wang, Y.-X. (2008). Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma atrum*. *Food Chemistry*, *107*(1), 231–241.

Chen, Y. G., Shen, Z. J., & Chen, X. P. (2009). Modulatory effect of *Ganoderma lucidum* polysaccharides on serum antioxidant enzymes activities in ovarian cancer rats. *Carbohydrate Polymers*, *78*(2), 258–262.

Chiang, P. D., Yen, C. T., & Mau, J. L. (2006). Non-volatile taste components of canned mushrooms. *Food Chemistry*, *97*(3), 431–437.

Cui, S. W. (2005). *Food carbohydrates: Chemistry, physical properties, and applications*. Boca Raton: Taylor & Francis Group/CRC Press.

Dalloul, R. A., & Lillehoj, H. S. (2006). Poultry coccidiosis: Recent advancements in control measures and vaccine development. *Expert Review of Vaccines*, *5*(1), 143–163.

Dong, C. H., & Yao, Y. J. (2008). In vitro evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of *Cordyceps sinensis*. *LWT – Food Science and Technology*, *41*(4), 669–677.

Dubost, N. J., Ou, B., & Beelman, R. B. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry*, *105*, 727–735.

Giannenas, I., Pappas, I. S., Mavridis, S., Kontopidis, G., Skoufos, J., & Kyriazakis, I. (2010). Performance and antioxidant status of broiler chickens supplemented with dried mushrooms (*Agaricus bisporus*) in their diet. *Poultry Science*, *89*(2), 303–311.

Guo, F. C., Savelkoul, H. F. J., Kwakkel, R. P., Williams, B. A., & Verstegen, M. W. A. (2003). Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. *Worlds Poultry Science Journal*, *59*, 427–440.

Hartman, S. (1998). *Calcium and selenium enrichment during cultivation improves the postharvest quality and shelf life of mushrooms* (*Agaricus bisporus*). MS Thesis. The Pennsylvania State University, University Park.

Hromadkova, Z., Ebringerova, A., & Valachovic, P. (2002). Ultrasound-assisted extraction of water-soluble polysaccharides from the roots of valerian (*Valeriana officinalis L.*). *Ultrasonics Sonochemistry*, *9*(1), 37–44.

Ionita, P. (2005). Is DPPH stable free radical a good scavenger for oxygen active species? *Chemical Papers*, *59*(1), 11–16.

Jeong, S. C., Jeong, Y. T., Yang, B. K., Islam, R., Koyyalamudi, S. R., Pang, G., et al. (2010). White button mushroom (*Agaricus bisporus*) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats. *Nutrition Research*, *30*(1), 49–56.

Jia, J., Zhang, X., Hu, Y.-S., Wu, Y., Wang, Q.-Z., Li, N.-N., et al. (2009). Evaluation of in vivo antioxidant activities of *Ganoderma lucidum* polysaccharides in STZ-diabetic rats. *Food Chemistry*, *115*(1), 32–36.

Kasuga, A., Aoyagi, Y., & Sugahara, T. (1995). Antioxidant activity of fungus *Suihs bovinus* (L: Fr.) O. Kuntze. *Journal of Food Science*, *60*(5), 1113–1115.

Lai, F. R., Wen, Q. B. A., Li, L., Wu, H., & Li, X. F. (2010). Antioxidant activities of water-soluble polysaccharide extracted from mung bean (*Vigna radiata* L.) hull with ultrasonic assisted treatment. *Carbohydrate Polymers*, *81*(2), 323–329.

Lai, L. S., & Yang, D. H. (2007). Rheological properties of the hot-water extracted polysaccharides in Ling-Zhi (*Ganoderma lucidum*). *Food Hydrocolloids*, *21*(5-6), 739–746.

Li, J., Fan, L., & Ding, S. (2011). Isolation, purification and structure of a new water-soluble polysaccharide from *Zizyphus jujuba* cv. *Jinsixiaozao*. *Carbohydrate Polymers*, *83*(2), 477–482.

Li, J. W., Ding, S. D., & Ding, X. L. (2007). Optimization of the ultrasonically assisted extraction of polysaccharides from *Zizyphus jujuba* cv. *jinsixiaozao*. *Journal of Food Engineering*, *80*(1), 176–183.

Liu, W., Xu, J., Jing, P., Yao, W., Gao, X., & Yu, L. (2010). Preparation of a hydroxypropyl *Ganoderma lucidum* polysaccharide and its physicochemical properties. *Food Chemistry*, *122*(4), 965–971.

Liu, Z. Q., Zhou, J. H., Zeng, Y. L., & Ouyang, X. I. (2004). The enhancement and encapsulation of *Agaricus bisporus* flavor. *Journal of Food Engineering*, *65*(3), 391–396.

Luo, A. X., He, X. J., Zhou, S. D., Fan, Y. J., Luo, A. S., & Chun, Z. (2010). Purification, composition analysis and antioxidant activity of the polysaccharides from *Dendrobium nobile* Lindl. *Carbohydrate Polymers*, *79*, 1014–1019.

Ma, J., Qiao, Z., & Xiang, X. (2011). Optimisation of extraction procedure for black fungus polysaccharides and effect of the polysaccharides on blood lipid and myocardium antioxidant enzymes activities. *Carbohydrate Polymers*, *84*(3), 1061–1068.

Matthaei, J. H., Jones, O. W., Martin, R. G., & Nirenberg, M. W. (1962). Characteristics and composition of RNA coding units. *Proceedings of the National Academy of Sciences of The United States of America*, *48*(4), 666–667.

Mayolo-Deloisa, K., Trejo-Hernandez, M. D., & Rito-Palomares, M. (2009). Recovery of laccase from the residual compost of *Agaricus bisporus* in aqueous two-phase systems. *Process Biochemistry*, *44*(4), 435–439.

Muralidhar, R. V., Chirumamilla, R. R., Ramachandran, V. N., Marchant, R., & Nigam, P. (2001). Racemic resolution of RS-baclofen using lipase from *Candida cylindracea* [Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen]. *Mededelingen*, *66*, 227–232.

Pan, Y., Hao, Y., Chu, T. W., Li, C. Q., Zhang, Z. F., & Zhou, Y. (2010). Ultrasonic-assisted extraction, chemical characterization of polysaccharides from Yunzhi mushroom and its effect on osteoblast cells. *Carbohydrate Polymers*, *80*(3), 922–926.

Qiao, D. L., Ke, C. L., Hu, B., Luo, J. G., Ye, H., Sun, Y., et al. (2009). Antioxidant activities of polysaccharides from *Hyriopsis cumingii*. *Carbohydrate Polymers*, *78*(2), 199–204.

Sun, Y. X., Li, T. B., & Liu, J. C. (2010). Structural characterization and hydroxyl radicals scavenging capacity of a polysaccharide from the fruiting bodies of *Auricularia polytricha*. *Carbohydrate Polymers*, *80*, 377–380.

Sun, Y. X., Liu, J. C., & Kennedy, J. F. (2010). Extraction optimization of antioxidant polysaccharides from the fruiting bodies of *Chroogomphis rutilus* (Schaeff.: Fr.) O.K. Miller by Box–Behnken statistical design. *Carbohydrate Polymers*, *82*(1), 209–214.

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.

Tseng, Y.-H., Yang, J.-H., & Mau, J.-L. (2008). Antioxidant properties of polysaccharides from *Ganoderma tsugae*. *Food Chemistry*, *107*(2), 732–738.

Turner, C., King, J. W., & Mathiasson, L. (2001). Supercritical fluid extraction and chromatography for fat-soluble vitamin analysis. *Journal of Chromatography A*, *936*, 215–237.

Vetter, J., & Lelley, J. (2004). Selenium level of the cultivated mushroom *Agaricus bisporus*. *Acta Alimentaria*, *33*, 297–301.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry – A review. *Innovative Food Science & Emerging Technologies*, *9*(2), 161–169.

Wang, B. (2011). Chemical characterization and Ameliorating effect of polysaccharide from Chinese jujube on intestine oxidative injury by ischemia and reperfusion. *International Journal of Biological Macromolecules*, *48*(3), 386–391.

Wang, J., Zhang, J., Zhao, B., Wang, X., Wu, Y., & Yao, J. (2010). A comparison study on microwave-assisted extraction of *Potentilla anserina* L. polysaccharides with conventional method: Molecule weight and antioxidant activities evaluation. *Carbohydrate Polymers*, *80*(1), 84–93.

Wang, L. C., Zhang, K., Di, L. Q., Liu, R., & Wu, H. (2011). Isolation and structural elucidation of novel homogenous polysaccharide from *Mactra veneriformis*. *Carbohydrate Polymers*, *86*(2), 982–987.

Wang, Y. J., Cheng, Z., Mao, J. W., Fan, M. G., & Wu, X. Q. (2009). Optimization of ultrasonic-assisted extraction process of *Poria cocos* polysaccharides by response surface methodology. *Carbohydrate Polymers*, *77*(4), 713–717.

Wu, X. J., & Hansen, C. (2008). Antioxidant capacity, phenolic content, and polysaccharide content of *Lentinus edodes* grown in whey permeate-based submerged culture. *Journal of Food Science*, *73*(1), M1–M8.

Wu, Y., Sun, C., & Pan, Y. (2006). Studies on isolation and structural features of a polysaccharide from the mycelium of an Chinese edible fungus (*Cordyceps sinensis*). *Carbohydrate Polymers*, *63*(2), 251–256.

Xie, J. H., Xie, M. Y., Nie, S. P., Shen, M. Y., Wang, Y. X., & Li, C. (2010). Isolation chemical composition and antioxidant activities of a water-soluble polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja. *Food Chemistry*, *119*, 1626–1632.

Xu, G., Zhang, H., & Hu, J. (2000). Leaching method of flavone from bamboo leaves. *Chinese Journal of Analytical Chemistry*, *28*, 857–859.

Xu, W. T., Zhang, F. F., Luo, Y. B., Ma, L. Y., Kou, X. H., & Huang, K. L. (2009). Antioxidant activity of a water-soluble polysaccharide purified from *Pteridium aquilinum*. *Carbohydrate Research*, *344*(2), 217–222.

Yang, B., Zhao, M. M., Shi, J., Yang, N., & Jiang, Y. M. (2008). Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. *Food Chemistry*, *106*(2), 685–690.

Ying, Z., Han, X., & Li, J. (2011). Ultrasound-assisted extraction of polysaccharides from mulberry leaves. *Food Chemistry*, *127*(3), 1273–1279.

Yu, R., Yang, W., Song, L., Yan, C., Zhang, Z., & Zhao, Y. (2007). Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris*. *Carbohydrate Polymers*, *70*(4), 430–436.

Yu, Z., LiHua, Y., Qian, Y., & Yan, L. (2009). Effect of *Lentinus edodes* polysaccharide on oxidative stress, immunity activity and oral ulceration of rats stimulated by phenol. *Carbohydrate Polymers*, *75*(1), 115–118.

Yuan, F., Yu, R. M., Yin, Y., Shen, J. R., Dong, Q. F., Zhong, L., et al. (2010). Structure characterization and antioxidant activity of a novel polysaccharide isolated from *Ginkgo biloba*. *International Journal of Biological Macromolecules*, *46*, 436–439.

Zhang, H., Ye, L., & Wang, K. (2010). Structural characterization and anti-inflammatory activity of two water-soluble polysaccharides from *Bellamya purificata*. *Carbohydrate Polymers*, *81*(4), 953–960.

Zhong, K., & Wang, Q. (2010). Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology. *Carbohydrate Polymers*, *80*, 19–25.

Zhu, M. Y., Wang, C. J., Gu, Y., He, C. S., Teng, X., Zhang, P., et al. (2009). Extraction, characterization of polysaccharides from *Morinda officinalis* and its antioxidant activities. *Carbohydrate Polymers*, *78*(3), 497–501.