Extraction of polysaccharides under vacuum condition from *Lentinus edodes* stipe and their antioxidant activities in vitro

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Abstract To improve the efficiency of polysaccharide extraction, vacuum technology was used to breakdown the cell wall to extract polysaccharides from *Lentinus edodes* stipe, and the optimal parameters were optimized by response surface methodology. Then the antioxidant activities in vitro of the polysaccharides were evaluated. Results showed that the optimal conditions for polysaccharide extraction were temperature of 62 °C, vacuum of 0.08 MPa, stirring speed of 1200 r/min, time of 25 min and material/liquid ratio of 1:26 (g/mL). Under these conditions, the yield of polysaccharide was 4.28%. The decolorization rate and deproteinization rate of polysaccharides treated by kaolin and hydrogen peroxide were higher than that of activated carbon. However, there was no significant influence on the polysaccharide retention rate, that was about 80%. Results also indicated that polysaccharide treated with activated carbon could obtain higher antioxidant activity.

Keywords *Lentinus edodes* stipe · Polysaccharide · Vacuum · Extraction · Antioxidant activity

Introduction

Lentinus edodes (also known as Xianggu or Shiitake mushroom), the most widely cultivated mushroom all over the world and the largest edible mushroom cultivated in China, is generally regarded as an edible and medical mushroom, which is characterized by a special fungal aroma and prevention capabilities against several diseases like cancer, diabetic, hypotensive, inflammatory, nociceptive, hypocholesterolemic (Bisen et al., 2010; Dermiki et al., 2013; Li et al., 2018; Rahman and Choudhury, 2012). According to the data of China Edible Fungi Association (2017), in 2016, the production of *Lentinus edodes* in China was 8.98 million tons, account for 25% of the total edible mushroom production in China.

Lentinus edodes stipes are the by-products of commercialization of mushroom processing, account for 20–30% of the whole fruitbody (Li et al., 2018; Wang et al., 2017). On the basis of the stipes as 20% of fruitbody, the output of *Lentinus edodes* stipes maybe reached up to 1.8 million tons. Owing to the high crude fiber content and poor palatability, most of shiitake mushroom stipes are usually discarded (Lin et al., 2008) except a little amount of them consumed by animals and/or processed to foods such as shiitake sauce, biscuits, bread and alcoholic beverage (Lin et al., 2010; Wang et al., 2017). Previous studies found that the stipe, as well as the cap, contains carbohydrates, proteins, amino acids, minerals, umami taste compounds, etc. (Chen et al., 2015; Li et al., 2018; Shi and Shao, 2003). Moreover, the findings also indicated that the *Lentinus edodes* stipe contains fiber and high chitin and its calcium content is also considerable in comparison to the cap (Li et al., 2018; On, 1976; Rahman and Choudhury, 2012; Yang et al., 2001; Yen and Mau, 2006, 2007). And the water holding capacity of the stipe powder of *Lentinus*

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edodes was higher than that of the cap powder (Zhang et al., 2012). In fact, *Lentinus edodes* stipe also contain active polysaccharides with the functions of enhancing immunity, preventing cancer and resisting cancer (Zheng and Ding, 1995). It is of great research value and practical significance to extract polysaccharide from the waste stipe with high efficiency. At present, hot-water extraction (Guo et al., 2001; Shi et al., 2009), enzymatic extraction (Ma et al., 2003), supersonic assisted extraction (Jiang, 2016), microwave extraction (Qin et al., 2017), radio frequency assisted extraction (Gao et al., 2009), etc. were commonly used to extract polysaccharide. However, these methods have some problems such as long extraction time, expensive equipment and unable to industrialization.

Vacuum extraction is a new type of extraction method by introducing a vacuum system into conventional extraction to forming micro-bubbles and blasting them instantaneously, causing strong cavitation effect around them and accelerating the dissolution of effective components in raw material tissues, thus realizing the purpose of rapid extraction at low temperature (Xu et al., 2011; Zhou et al., 2015). Vacuum technology has achieved some results in the extraction of total flavonoids from *Ginkgo* leaves (Zhou et al., 2015), chlorogenic acid from honeysuckle (Xu et al., 2011), astaxanthin from *Phaffia rhodozyma* (Zu et al., 2007), tannic acid from Chinese gallnut (Min et al., 2013), proanthocyanidins from *Ginkgo biloba* leaves (Wang et al., 2013), polyphenols from *Rhus verniciflua* stokes (Chen et al., 2015), etc. Therefore, vacuum combined with ultrasound extraction on polyphenols from apple pomace (Tian et al., 2017), vacuum combined with microwave extraction on flavonoids from onion (Zill-e-Huma et al., 2011), vitamin C from guava and green pepper and vitamin E from soybean and tea leaves (Xiao et al., 2009), polyphenolic compounds and pigment from Chinese herbs (Wang et al., 2008), have also achieved better results. However, there are no related reports on polysaccharides extraction from edible fungi using vacuum technology. The objective of this study was to breakdown the cell wall using vacuum technology to improve the yield of polysaccharide from *Lentinus edodes* stipe, and to evaluate the antioxidant activities in vitro of the decolorized and undecolorized polysaccharides.

Materials and methods

Materials

Dried *Lentinus edodes* stipe was purchased from a local market in Zhengzhou, Henan Province, China. The dried stipes were grinded by high-speed pulverizer (300Y, Yongkang Boou Hardware Products Co., Ltd, Yongkang,

Zhejiang Province, China) for 1 min, and screened through 40 mesh sized sieve before use.

Extraction of polysaccharides

Five grams of dried *Lentinus edodes* stipe powders were extracted with distilled water under vacuum conditions and extracted experimentally at a variety of selected conditions under different temperatures (30, 40, 50, 60, 70 °C), stirring speed (0, 800, 1200, 1600, 2400 r/min), material/liquid ratio (1:5, 1:10, 1:15, 1:20, 1:25, 1:30 g/mL), vacuum (0.00, 0.02, 0.04, 0.06, 0.08 MPa) and extraction time (5, 10, 15, 20, 25, 30 min). After centrifugation at 1700×*g* for 10 min, the supernatant was concentrated using a vacuum concentrator (RV 8 V-C, IKA, Germany). The polysaccharide was precipitated with 3 times volume of anhydrous ethanol for 12 h, then centrifuged at 1700×*g* for 10 min, and the precipitate was dissolved in distilled water. The polysaccharide content was determined by phenol–sulfuric acid method (Dubois et al., 1956).

Based on the results of single-factor experiments, the optimal conditions for vacuum extraction were determined by response surface methodology (RSM), with the standard of five-levels, four-factors central composite design (CCD) designed by Design-Expert® 11.0.4.0 (Stat-Ease Inc., Minneapolis, MN, USA). Table 1 shows the range and center point values of the four independent variables based on the results of the above single factor experiments. The CCD in the experimental design consisted of 30 treatments.

Decolorization

Decolorization rate of polysaccharide extracted from *Lentinus edodes* stipe decolorized by activated carbon, kaolin and hydrogen peroxide were measured as described by Li and Chen (2016). Decolorization rate was measured at 420 nm. Protein content and polysaccharide content were determined according to Bradford (1976) and Dubois et al. (1956), respectively.

Antioxidant activity of polysaccharides

Assay of DPPH radicals, hydroxyl radicals scavenging activities and reducing power

Scavenging activities on DPPH radicals and hydroxyl radicals, reducing power of polysaccharide extracted from *Lentinus edodes* stipe were measured according to the description of Tsai et al. (2006).

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

Variables	Coded variables	Levels				
		− 2	− 1	0	1	2
Extraction temperature (°C)	X_1	40	50	60	70	80
Stirring speed (r/min)	X_2	400	800	1200	1600	2000
Material/liquid ratio (g/mL)	X_3	1:15	1:20	1:25	1:30	1:35
Extraction time (min)	X_4	15	20	25	30	35

Assay of $ABTS^+$ radicals scavenging activity

Measurements were done according to Darré et al. (2017) using the $ABTS^+$ (2, 20-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) method with slight modification. The stock solution of stable radical-cation $ABTS^+$ was prepared using 7 mmol/L of the ammonium salt of $ABTS^+$ in 2.45 mmol/L $K_2S_2O_8$ at a ratio of 2:1 (V/V) and left overnight at room temperature before use. An ethanolic dilution was performed to reach an absorbance of 0.700 ± 0.02 at 734 nm. A 0.1 mL of polysaccharide solution was mixed with 1 mL of $ABTS^+$ diluted solution, vortexed and the absorbance at 734 nm was measured after 6 min.

Statistical analysis

All analyzes were performed in triplicate. Experimental data were analyzed using analysis of variance (ANOVA) with SPSS statistical software. Data were presented as mean $\pm$ standard error (SE) using Duncan's test ($p < 0.05$). Analysis of response surface were performed using the software Design-Expert® 11.0.4.0 (Stat-Ease Inc., Minneapolis, MN, USA).

Results and discussion

Single-factor experiment analysis

Effect of extraction temperature on the yield of polysaccharide

As an important factor that affect the yield of polysaccharide, different extraction temperatures (30, 40, 50, 60, 70 °C) on the yield of polysaccharide were evaluated when the stirring speed, material/liquid ratio, vacuum and extraction time were set at 2400 r/min, 1:10 (g/mL), 0.08 MPa and 10 min, respectively. As shown in Fig. 1A, the yield of polysaccharide increased from 1.84 to 2.54% when the temperature increased from 30 to 60 °C, then decreased from 60 to 70 °C. This result was in accordance with that of Huang et al. (2015), Wang et al. (2016) and Yin et al. (2018). This phenomenon may due to the higher

temperature caused the degradation of polysaccharides (Huang et al., 2015; Wang et al., 2016). Therefore, 60 °C was considered to be the optimal extraction temperature.

Effect of stirring speed on the yield of polysaccharide

Different stirring speed (0, 800, 1200, 1600, 2400 r/min) were studied with extraction temperature of 60 °C, material/liquid ratio of 1:10 (g/mL), vacuum of 0.08 MPa and extraction time of 10 min. The effect of different stirring speed on the yield of polysaccharide of *Lentinus edodes* stipe was shown in Fig. 1B. The yield of polysaccharide increased significantly with the increase of stirring speed and then decreased slightly when the stirring speed exceeded 1200–1600 r/min. A possible reason for this result was that the stirring under vacuum condition can increase the bubbles, and then fortified the cavitation effects (Zhou et al., 2015). Thus, 1200 r/min was selected as an optimal stirring speed.

Effect of material/liquid ratio on the yield of polysaccharide

Under the conditions as extraction temperature of 60 °C, stirring speed of 1200 r/min, vacuum of 0.08 MPa and extraction time of 10 min, the effect of material/liquid ratio on the yield of polysaccharide of *Lentinus edodes* stipe was investigated. As shown in Fig. 1C, with the increase of material/liquid ratio, more polysaccharide was extracted due to the power of mass transfer increased. The yield of polysaccharide reached 2.65% at material/liquid ratio of 1:25 (g/mL). When this ratio was increased, a slight decrease of the yield of polysaccharide was observed. In this study, 1:25 (g/mL) was selected as the appropriate material/liquid ratio.

Effect of vacuum on the yield of polysaccharide

To clarify vacuum effect on the yield of polysaccharide, different vacuums (0, 0.02, 0.04, 0.06 and 0.08 MPa) were set while the extraction temperature, stirring speed, material/liquid ratio, and extraction time were set at 60 °C, 1200 r/min, 1:25 (g/mL) and 10 min, respectively. Figure 1D showed that, in the range of the experiment, the

Fig. 1 Effect of different extraction temperature (A), stirring speed (B), material/liquid ratio (C), vacuum (D) and extraction time (E) on the yield of polysaccharides from *Lentinus edodes* stipe

yield of polysaccharide increased markedly with the increase of vacuum. The yield of polysaccharide reached 3.46% at vacuum of 0.08 MPa, being 1.22 times higher than that at 0 MPa. This result was similar to that of Huang et al., (Huang et al., 2015) on ultrasonic-vacuum extraction of polysaccharide from *Pleurotus eryngii* and Zhou et al., (Zhou et al., 2015) on vacuum extraction of total flavonoids from *Ginkgo* leaves. This may be due to the fact that the boiling point of the water become lower with the increase of vacuum. It produced more bubbles and the cavitation effect become stronger, then promoted mass-liquid exchange, and finally increased the yield of polysaccharide. So, vacuum of 0.08 MPa was considered to be the optimal condition in this study.

Effect of extraction time on the yield of polysaccharide

To evaluate the effect of extraction time on the yield of polysaccharide, experiments were performed under the fixed conditions (extraction temperature of 60 °C, stirring speed of 1200 r/min, material/liquid ratio of 1:25 (g/mL) and vacuum of 0.08 MPa). The results showed that as the extraction time prolonged, the yield of polysaccharide increased significantly, and reached 3.64% at 25 min, then decreased (Fig. 1E). This result indicated that the yield of polysaccharide was closely related with extraction time. The shorter time resulted in insufficient extraction, brought about the lower yield of polysaccharide. Oppositely, the longer time may breakdown the structure of polysaccharide and reduce the yield of polysaccharide (Huang et al., 2015; Zhou et al., 2015). To obtain a higher yield of polysaccharide, 25 min was selected as the appropriate extraction time.

RSM optimization

Model fitting

Based on the results of single factors experiments, extraction temperature, stirring speed, vacuum and extraction time were selected to optimize the yield of polysaccharide by RSM using CCD. 24 runs with 6 center points were carried out randomly and the results are shown in Table 2. By applying multiple regression analysis on the experimental data, the response and test variables were found to correlated with quadratic polynomial equation as follows:

$$Y = 4.08 + 0.22X_1 + 0.047X_2 + 0.17X_3 + 0.068X_4 + 0.016X_1X_2 + 0.11X_1X_3 - 0.13X_1X_4 - 0.17X_2X_3 - 0.062X_2X_4 - 0.11X_3X_4 - 0.60X_1^2 - 0.41X_2^2 - 0.43X_3^2 - 0.43X_4^2$$

where Y is the yield of polysaccharide extracted from *Lentinus edodes* stipe (%); X_1 , X_2 , X_3 and X_4 are the coded variables of extraction temperature, stirring speed, material/liquid ratio and extraction time, respectively.

The analysis of variance (ANOVA) for the response surface quadratic model was shown in Table 3. The low p value ($p < 0.0001$) of the model indicated that it was highly statistically significant. The p -value (0.0969) of lack-of-fit indicated that it was insignificant ($p > 0.05$) relative to the pure error. It means that the quadratic model was adequate to predict the variations. The determination coefficient (R^2) and adjusted determination coefficient ($-R^2_{Adj}$) had a value of 0.9398 and 0.8836, respectively. This indicated that the model was acceptable in terms of experimental errors and reliable for the prediction of the yield of polysaccharide. In this case, the independent variables (X_1 and X_3), the interaction term (X_2X_3), and all the quadratic terms (X_1^2 , X_2^2 , X_3^2 and X_4^2) significantly affected the yield of polysaccharide.

Interaction between variables

Tri-dimensional response surface plots were constructed to illustrate the relationship between responses and the levels of the processing variables, and the interactions between two variables with the other factors were set at zero (Fig. 2). Figure 2A showed the effects of extraction temperature (X_1) and stirring speed (X_2) on the yield of polysaccharide. With the material/liquid ratio set at 1:25 (g/mL) and the extraction time at 25 min, the optimal yield of polysaccharide should be found with a temperature range of 57.80–65.95 °C with stirring speed between 1029.07 r/min and 1418.68 r/min. Figure 2B represented the effects of temperature (X_1) and material/liquid ratio (X_3) on the yield of polysaccharide. When the stirring speed and extraction time set at 1200 r/min and 25 min, respectively, the optimal yield of polysaccharide was observed at a temperature of 57.56–66.58 °C and the material/liquid ratio of 1:(23.46–28.75) (g/mL). Figure 2C showed the interaction of temperature (X_1) and extraction time (X_4) on the yield of polysaccharide. When the stirring speed set at 1200 r/min and the material/liquid ratio at 1:25 (g/mL), the highest yield of polysaccharide was found with a temperature of 57.70–65.93 °C and an extraction time of 22.83–27.68 min. Figure 2D represented the effects of stirring speed (X_2) and material/liquid ratio (X_3) on the yield of polysaccharide. The yield of polysaccharide initially increased with the increase of stirring speed and

Table 2 Experimental design and results of response surface analysis

No.	X_1	X_2	X_3	X_4	Yield of polysaccharides (%)	
					Measured	Predicted
1	−1	−1	1	−1	2.12	2.03
2	−1	1	1	−1	1.96	1.88
3	0	0	0	2	2.23	2.50
4	−1	−1	−1	1	2.22	2.10
5	2	0	0	0	1.59	2.14
6	0	2	0	0	2.51	2.53
7	1	1	1	1	2.51	2.37
8	0	0	0	0	4.08	4.08
9	−2	0	0	0	1.36	1.25
10	0	0	2	0	2.35	2.68
11	1	1	−1	−1	2.44	2.40
12	1	1	−1	1	2.57	2.37
13	0	0	0	0	4.34	4.08
14	−1	1	−1	1	2.37	2.38
15	−1	1	−1	−1	1.78	1.89
16	0	−2	0	0	1.93	2.34
17	1	−1	−1	−1	2.15	1.81
18	−1	−1	−1	−1	1.35	1.36
19	0	0	0	0	4.00	4.08
20	0	0	−2	0	1.91	2.01
21	0	0	0	0	4.22	4.08
22	1	−1	1	1	3.11	2.71
23	1	−1	1	−1	3.08	2.93
24	−1	1	1	1	1.89	1.93
25	1	−1	−1	1	2.08	2.03
26	−1	−1	1	1	2.43	2.33
27	0	0	0	0	3.81	4.08
28	1	1	1	−1	3.02	2.84
29	0	0	0	0	4.01	4.08
30	0	0	0	−2	2.07	2.23

Table 3 ANOVA for response surface quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	20.67	14	1.48	16.73	< 0.0001
X_1	1.18	1	1.18	13.36	0.0023
X_2	0.0540	1	0.0540	0.6121	0.4462
X_3	0.6755	1	0.6755	7.65	0.0144
X_4	0.1100	1	0.1100	1.25	0.2817
X_1X_2	0.0041	1	0.0041	0.0467	0.8319
X_1X_3	0.2030	1	0.2030	2.30	0.1501
X_1X_4	0.2802	1	0.2802	3.18	0.0950
X_2X_3	0.4578	1	0.4578	5.19	0.0378
X_2X_4	0.0625	1	0.0625	0.7084	0.4132
X_3X_4	0.1919	1	0.1919	2.17	0.1610
X_1^2	9.73	1	9.73	110.28	< 0.0001
X_2^2	4.63	1	4.63	52.45	< 0.0001
X_3^2	5.16	1	5.16	58.48	< 0.0001
X_4^2	5.00	1	5.00	56.66	< 0.0001
Residual	1.32	15	0.0882		
Lack of Fit	1.15	10	0.1152	3.36	0.0969
Pure Error	0.1717	5	0.0343		

$$R^2 = 0.9398, R^2_{\text{Adj}} = 0.8836, C.V. \% = 11.50$$

Fig. 2 Tri-dimensional response surface plots showing the experimental factors and their mutual interactions on the yield of polysaccharides extracted from *Lentinus edodes* stipe. **A** $Y = f(X_1, X_2, 25, 25)$; **B** $Y = f(X_1, 1200, X_3, 25)$; **C** $Y = f(X_1, 1200, 25, X_4)$;

D $Y = f(60, X_2, X_3, 25)$; **E** $Y = f(60, X_2, 25, X_4)$; **F** $Y = f(60, 1200, X_3, X_4)$. Y , yield of polysaccharides (%); X_1 , extraction temperature (°C); X_2 , stirring speed (r/min); X_3 , material/liquid ratio (g/mL); X_4 , extraction time (min)

material/liquid ratio, then gradually declined. With the temperature set at 60 °C and the extraction time at 25 min, the optimal yield of polysaccharide was found under stirring speed and material/liquid ratio ranges of 1013.86–1401.48 r/min and 1:(23.58–28.31) (g/mL), respectively. Figure 2E, F showed the effects of stirring speed (X_2) and extraction time (X_4), material/liquid ratio (X_3) and extraction time (X_4) on the yield of polysaccharide. The maximum yield of polysaccharide was achieved when stirring speed of 1042.80–1396.86 r/min and extraction time of 23.19–27.56 min, material/liquid ratio of 1:(23.59–28.59) (g/mL) and extraction time of 22.90–27.64 min, respectively.

Variables optimization and model validation

The optimal values of the four studied variables obtained from the quadratic model were 62 °C, 1200 r/min, 1:26 (g/mL) and 25 min of extraction temperature, stirring speed, material/liquid ratio and extraction time, respectively. The model predicted maximum extraction yield of polysaccharide was 4.12% under the optimal conditions. For validation of the suitability of the model, triplicate confirmatory experiments were performed, and the average yield of polysaccharide was (4.28 ± 0.18) %. This result was lower than that of microwave assisted extraction (microwave power of 400 W, material/liquid ratio of 1:7 g/mL, extraction time of 8 min, the yield of polysaccharide was 4.91%) of polysaccharide from *Lentinus edodes* stipe (Qin et al., 2017), higher than that of hot-water extraction (temperature of 98 °C, material/liquid ratio of 1:20 g/mL, extraction time of 90 min, times of 3 extraction, the yield of polysaccharide was 3.64%) (Guo et al., 2001). This result indicated that the constructed quadratic model was reliable to predict the yield of polysaccharide extracted from *Lentinus edodes* stipe.

Comparison the efficiency of different decolorization methods

The effects on polysaccharide retention rate, deproteinization rate and decolorization of polysaccharide extracted from *Lentinus edodes* stipe treated with activated carbon, kaolin and hydrogen peroxide were shown in Fig.S1. The results indicated that there was no significant difference on polysaccharide retention rate among treatments, and the polysaccharide retention rate in all treatments was above 80%. However, a lowest deproteinization rate and decolorization rate (32.59% and 64.25%) were observed in activated carbon compared with kaolin (61.21% and 73.51%) and hydrogen peroxide (60.57% and 93.28%). From the point of polysaccharide purification, kaolin or hydrogen peroxide should be selected. This result

was higher than that of Li and Chen (2016) on sweet potato leaf.

Antioxidant activity of polysaccharide extracted from *Lentinus edodes* stipe

DPPH radicals scavenging activity

As can be seen from Fig. 3A, the DPPH radicals scavenging abilities of the four polysaccharides increased with the increase of polysaccharide concentration, showing a dose–effect relationship. However, the scavenging rate of all four polysaccharides was lower than 50%. Within the experimental concentration range, the DPPH scavenging rate of vitamin C (VC) was remained at about 20%. The DPPH radicals scavenging rates of all polysaccharides, except for the hydrogen peroxide treatment, were higher than VC. The polysaccharides decolorized by activated carbon had the highest DPPH radicals scavenging rate, that reached 43.58% when the polysaccharide concentration was 2 mg/mL, while the polysaccharide extract and polysaccharides decolorized by kaolin and hydrogen peroxide under the same concentration were 38.68, 37.27 and 30.31%, respectively.

Hydroxyl radicals scavenging activity

Fenton reaction was used to detect the elimination of hydroxyl radicals ($\cdot\text{OH}$) of the polysaccharides extracted from *Lentinus edodes* stipe, and the results were shown in Fig. 3B. It can be seen from Fig. 3B that the $\cdot\text{OH}$ scavenging rates of all the four polysaccharides were lower than that of VC. All the four polysaccharides except polysaccharide decolorized by hydrogen peroxide can effectively remove $\cdot\text{OH}$, and the scavenging rate increased with the increase of the concentration of polysaccharide. When the polysaccharide concentration was less than 1.0 mg/mL, there was no significant difference ($p > 0.05$) among polysaccharide extract and polysaccharide decolorized by activated carbon and kaolin. With the polysaccharide concentration increased to 2 mg/mL, the $\cdot\text{OH}$ scavenging rate of polysaccharide extract reached 88.67%, which was significantly higher than that of polysaccharides decolorized by activated carbon and kaolin (53.52% and 50.78%, respectively).

Reducing power

Figure 3C showed that the reducing power of the four polysaccharides except for polysaccharide treated with hydrogen peroxide increased with the increase of polysaccharide concentration, but their reducing power was lower than that of VC. The polysaccharide treated with

Fig. 3 Antioxidant activities of polysaccharide extract, polysaccharide decolorized by activated carbon, kaolin and hydrogen peroxide, and vitamin C (Vc). **A** DPPH radicals scavenging activity; **B** hydroxyl

radicals scavenging activity; **C** reducing power; **D** ABTS⁺ radicals cation scavenging activity

hydrogen peroxide showed almost no reducing power, and the changes of reducing power of polysaccharide treated with activated carbon and kaolin was consistent with the trend of polysaccharide extract.

ABTS⁺ radicals scavenging activity

It can be seen from Fig. 3D that the ABTS⁺ radicals scavenging capacity was positively correlated with the polysaccharide concentration. In general, the ABTS⁺ radicals scavenging capacity of the polysaccharides with the same concentration was lower than that of VC. Compared with polysaccharide extract, polysaccharide treated with activated carbon treatment could significantly improve the scavenging ability of ABTS⁺ radicals, while polysaccharide treated with kaolin and hydrogen peroxide showed a lower ABTS⁺ radicals scavenging ability than that of polysaccharide extract.

Based on the above results, it can be concluded that polysaccharide treated with hydrogen peroxide has almost lost its antioxidant activity. Although the polysaccharide

treated with activated carbon showed a poorer deproteinization rate and decolorization rate than polysaccharide treated with kaolin and hydrogen peroxide (Fig. S1). However, under the same polysaccharide concentration, the antioxidant activity of polysaccharide treated with activated carbon was higher than those of polysaccharide extract and polysaccharide treated with kaolin and hydrogen peroxide.

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References

- Andō A. On the components of pileus and stipe of dried shiitake (*Lentinus edodes* (Berk.) Sing): chemical components and hot water soluble polysaccharides. *J. Home Econ. Jpn* 27: 86–91 (1976)

- Bisen PS, Baghel RK, Sanodiya BS, Thakur GS, Prasad GBKS. *Lentinus edodes*: a macrofungus with pharmacological activities. *Curr. Med. Chem.* 17: 2419–2430 (2010)
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254 (1976)
- Chen W, Li W, Yang Y, Yu H, Zhou S, Feng J, Li X, Liu Y. Analysis and evaluation of tasty components in the pileus and stipe of *Lentinula edodes* at different growth stages. *J. Agric. Food Chem.* 63: 795–801 (2015)
- Chen H, Wang C, Ye J, Zhou H, Zhang Y, Tao R. Phenolic extracts from *Rhus verniciflua* stokes woods by vacuum extraction and their anti-tyrosinase activities. *Chem. Ind. Forest Prod.* 35: 47–54 (2015)
- China Edible Fungi Association. Notice on the results of 2016 annual output, output value and export statistics of edible fungus in China (China Edible Fungi Association [2017]11). Beijing (2017). Available from: <http://www.cefa.org.cn/2017/10/24/10250.html>. Accessed 24 Oct 2017
- Darré M, Valera L, Ortiz Araque LC, Lemoine ML, Demkura PV, Vicente AR, Concellón A. Role of UV-B irradiation dose and intensity on color retention and antioxidant elicitation in broccoli florets (*Brassica oleracea* var. *Italica*). *Postharvest Biol. Tech.* 128: 76–82 (2017)
- Dermiki M, Phanphensophon N, Mottram DS, Methven L. Contributions of non-volatile and volatile compounds to the umami taste and overall flavour of shiitake mushroom extracts and their application as flavour enhancers in cooked minced meat. *Food Chem.* 141: 77–83 (2013)
- Dubois M, Gilles K A, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350–356 (1956)
- Gao H, Wang Y, Shi D, Wang S, He J, Guan J. Radio-frequency assisted extraction for polysaccharides from stems of shiitake mushroom. *Trans. CSAE.* 25: 180–184 (2009)
- Guo D, Ruan C, Liu H. Study on the extraction of water soluble polysaccharide from dried mushroom stem J. Heilongjiang Aug First Land Reclam Univ 13: 78–81 (2001)
- Huang Q, Yue T, Yuan Y, Wang Z. Optimization of ultrasonic-vacuum extraction of polysaccharides from fruit bodies of *Pleurotus eryngii*. *Food Sci.* 36: 77–82 (2015)
- Jiang Y. On the technology of ultrasonic-assisted polysaccharide extraction of shii-take stipe. *Val. Eng.* 3: 137–139 (2016)
- Li F, Chen L. Decoloring research on sweet potato leaf. *J. Org. Chem. Res.* 4: 82–92 (2016)
- Li S, Wang A, Liu L, Tian G, Wei S, Xu F. Evaluation of nutritional values of shiitake mushroom (*Lentinus edodes*) stipes. *J. Food Meas. Charact.* 12: 2012–2019 (2018)
- Lin L-Y, Tseng Y-H, Li R-C, Mau J-L. Quality of shiitake stipe bread. *J. Food Process. Pres.* 32: 1002–1015 (2008)
- Lin P-H, Huang S-Y, Mau J-L, Liou B-K, Fang T J. A novel alcoholic beverage developed from shiitake stipe extract and cane sugar with various *Saccharomyces* strains. *LWT Food Sci. Technol.* 43: 971–976 (2010)
- Ma C, Xia R, Peng Y, He X. A study of methods for extraction of lentinan and amino acids from the foot body of *Lentinus edodes*. *Herald Med.* 22: 372–374 (2003)
- Min F, Zhang Z, Qin Q, Ye J, Wang C. Optimization of vacuum cavitation extraction of tannic acid from Chinese gallnut using response surface method. *Nat. Prod. Res. Dev.* 25: 1240–1244 (2013)
- Qin N, Miao W-Y, Lian W-Q, Sun Y-L. Optimization of microwave-assisted extraction of polysaccharides from *Lentinus edodes* root by response surface analysis and its antioxidant activity. *J. Food Saf. Qual.* 8: 594–600 (2017)
- Rahman T, Choudhury M. Shiitake mushroom: A tool of medicine. *Bangladesh J. Med. Biochem.* 5: 24–32 (2012)
- Shi Q, Shao W. Determination of nutritive components of eight edible fungi. *J. Gansu Agric. Univ.* 38: 223–339 (2003)
- Shi D, Gao H, Zhou M, Cheng W, Wang S, Du X, Yang D. Effect of super fine crushing on the extraction yield of polysaccharide in *Lentinus edodes* stem. *Hubei Agric. Sci.* 48: 1730–1732 (2009)
- Tian L, Li H, Yuan Y, Yue T, Wei J. Optimization of ultrasound-vacuum assisted extraction of polyphenols from apple pomace. *Food Sci.* 38: 233–239 (2017)
- Tsai S-Y, Huang S-J, Mau J-L. Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. *Food Chem.* 98: 670–677 (2006)
- Wang J-X, Xiao X-H, Li G-K. Study of vacuum microwave-assisted extraction of polyphenolic compounds and pigment from Chinese herbs. *J. Chromatogr. A* 1198–1199: 45–53 (2008)
- Wang Y, Xu L, Cao F, Wang W, Zhao F, Yang S, Lei M, Chen Y, Tang Y, Jiang G, Wang G. Negative pressure cavitation-assisted extraction and purification of proanthocyanidins from *Ginkgo biloba* leaves. *Food Sci.* 34: 6–10 (2013)
- Wang L, Cheng L, Xu Y, Wang T, Li L, Wang H. Extraction technology optimization and antioxidant activity of polysaccharides from pumpkin seeds. *Trans. CSAE.* 32(9): 284–290 (2016)
- Wang A, Li J, Wang X, Li S, Liu L, Tian G. Decolorization of dietary fiber from fermented *Lentinus edodes* stipe by the combination of ultrasound and H₂O₂. *Packag. Food Mach.* 35: 19–23 (2017)
- Xiao X-H, Wang J-X, Wang G, Wang J-Y, Li G-k. Evaluation of vacuum microwave-assisted extraction technique for the extraction of antioxidants from plant samples. *J. Chromatogr. A* 1216: 8867–8873 (2009)
- Xu L, Xu G, Wang Z, Xiao W. Study on vacuum extraction process of honeysuckle. *Chinese J. Exp. Tradit. Med. Formulae* 17: 31–33 (2011)
- Yang J-H, Lin H-C, Mau J-L. Non-volatile taste components of several commercial mushrooms. *Food Chem.* 72: 465–471 (2001)
- Yen M-T, Mau J-L. Preparation of fungal chitin and chitosan from shiitake stipes. *Fungal Sci.* 21: 1–11 (2006)
- Yen M-T, Mau J-L. Selected physical properties of chitin prepared from shiitake stipes. *LWT Food Sci. Technol.* 40: 558–563 (2007)
- Yin C, Fan X, Fan Z, Shi D, Gao H. Optimization of enzymes-microwave-ultrasound assisted extraction of *Lentinus edodes* polysaccharides and determination of its antioxidant activity. *Int. J. Biol. Macromol.* 111: 446–454 (2018)
- Zhang Z, Song H, Peng Z, Luo Q, Ming J, Zhao G. Characterization of stipe and cap powders of mushroom (*Lentinus edodes*) prepared by different grinding methods. *J. Food Eng.* 109: 406–413 (2012)
- Zheng J, Ding X. Study on the antimutative PJF1 polysaccharide from foot body of *Lentinus edodes*. *J. Wuxi Univ. Light Ind.* 14: 7–13 (1995)
- Zhou H, Wang C, Ye J, Chen H, Tao R, Zhang Y. Optimization of decompressing ebullition extraction of total flavonoids from *Ginkgo* leaves. *J. Northeast For. Univ.* 43: 128–132 (2015)
- Zill-e-Huma, Abert-Vian M, Elmaataoui M, Chemat F. A novel idea in food extraction field: Study of vacuum microwave hydrodiffusion technique for by-products extraction. *J. Food Eng.* 105: 351–360 (2011)
- Zu Y, Liu L, Xue Y, Shi Q. Extraction of astaxanthin by vacuum cavitation. *J. Northeast For. Univ.* 35: 59–60 (2007)