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Short communication

Drying effects on the antioxidant properties of polysaccharides obtained from *Agaricus blazei Murrill*

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ABSTRACT

Three polysaccharides (ABMP-F, ABMP-V, ABMP-A) were obtained from *Agaricus blazei Murrill* via methods such as freeze drying, vacuum drying and air drying, respectively. Their chemical compositions were examined, and antioxidant activities were investigated on the basis of assay for hydroxyl radical, DPPH radical, ABTS free radical scavenging ability and assay for Fe^{2+}-chelating ability. Results showed that the three ABMPs have different physicochemical and antioxidant properties. Compared with air drying and vacuum drying methods, freeze drying method resulted to ABMP with higher neutral sugar, polysaccharide yield, uronic acid content, and stronger antioxidant abilities of hydroxyl radical, DPPH radical, ABTS radical scavenging and Fe^{2+}-chelating. As a result, *Agaricus blazei Murrill* polysaccharides are natural antioxidant and freeze drying method serves as a good choice for the preparation of such polysaccharides and should be used to produce antioxidants for food industry.

1. Introduction

Agaricus blazei Murrill (ABM) is a kind of mushroom which has long been used as a folk remedy for curing various diseases such as cancers, heart disease and diabetes in Brazil and Japan. Polysaccharide is one of the main bioactive constituents of *Agaricus blazei Murrill* with health functions (Delmanto et al., 2001; Mizuno, 1995). In order to realize all its promising applications, there is a great demand to supply the market with high-quality *Agaricus blazei Murrill* polysaccharides (ABMP).

It is generally admitted that free radicals which cause lipid peroxidation, decrease permeation, damage membrane proteins and contribute to cellular inactivation (Borchani, Besbes, Masmoudi, Blecker, Paquot, & Attia, 2011). Antioxidant properties of polysaccharides are reflected in improving the activity of antioxidant enzymes, scavenge free radicals and inhibit lipid peroxidation (Xu, Liu, Yao, Pang, Yin, & Gao, 2009). Dehydration is one of the most important preservation methods employed in the storage of mushroom and dehydrated mushrooms are used in sauces and soups widely (Giri & Prasad, 2007). Various drying methods have been developed to preserve food including mushrooms and have exhibited their own characteristics. Thus, it is necessary to evaluate the influence of drying methods on antioxidant activities of polysaccharides obtained from ABM.

The studies on drying methods and processing treatment on ABM were limited. The impact of drying methods on physicochemical properties and antioxidant activities of ABMP are still unknown. The aims of the present study are, therefore, to examine the chemical composition and antioxidant activities of ABMP treated by different drying methods (air, vacuum, and freeze drying) for seeking the most proper drying method for the production of active polysaccharides.

2. Materials and methods

2.1. Materials

Agaricus blazei Murrill (ABM) were purchased from a local market in Yunnan, China. Samples were ground and passed through 80 mesh screen.

D-Glucose was obtained from Tianjin Zhi En-Biotech-nological Co., Ltd. Glucuronic acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2-azino-bis-(3-ethyl-benzthia-6-sulfonic acid) (ABTS), and ferrozine were purchased from Heowns Biochem Technologies LLC. All chemicals were reagent grade or better.

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2.2. Polysaccharides extracted from Agaricus blazei Murrill

Polysaccharides were extracted with hot water (1: 30 ratio of raw material to water, w/v) for 3 h at 95 °C. The extract was filtered and the filtrate was condensed in a rotary evaporator under reduced pressure at 60 °C, and was then precipitated by adding ethanol (12 h, 4 °C) to a final concentration of 80% (v/v). The precipitates were collected by centrifugation (4200 rpm, 15 min) and washed three times by dehydrated alcohol. Then the precipitates were lyophilized to obtain crude polysaccharides.

2.3. Drying procedure

Drying experiment of crude ABMP was conducted via three approaches including freeze drying (FD), vacuum drying (VD) and air drying (AD). FD was done at −60 °C in vacuum freeze dryer. VD was done at 60 °C and 0.09 MPa of vacuum degree. AD was done in the air at room temperature.

2.4. Analytical methods

The polysaccharides content was measured by phenol–sulfuric acid method using D-glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The percentage ABMP extraction yield (%) was calculated with the formula of $y(\%) = c/w \times 100$, where c was the polysaccharides content of extraction, and w represented dried sample weight (1 g). The protein content was determined by using Bradford's method (Thongboonkerd, Chutipongtanate, & Kanlaya, 2006), with bovine serum albumin (BSA) as standard. The uronic acid content was determined by 3-hydroxydiphenyl assay using glucuronic acid as reference material (Blumenkrantz & Asboe-Hansen, 1973).

2.5. Antioxidant properties

2.5.1. Assay for DPPH radical scavenging activity

The radical scavenging activity of ABMP was conducted by using the method previously carried out by Ma, Chen, Zhu, & Wang (2011). Briefly, 2 mL of DPPH solution was added into 2 mL ABMP solution at a concentration ranging from 0 to 1.0 mg/mL. The mixture was placed in the darkness for 30 min and then the absorbance was measured at 517 nm.

2.5.2. Assay for hydroxyl radical scavenging activity

The hydroxyl radical scavenging assay was performed on the basis of a literature procedure with a few modifications (Fan et al., 2011). The reaction mixture consisted of 1.0 mL $FeSO_4$ (3.5 mM), 0.7 mL H_2O_2 (6 mM), 1.0 mL of series of concentrations ABM extracts and 0.3 mL sodium salicylate (20 mM). The total mixture was incubated at 37 °C for 1 h and the absorbance was measured at 562 nm.

2.5.3. Total antioxidant activity

The total radical scavenging capacity was assessed with a reported procedure (Wang, Chang, Inbaraj, & Chen, 2010) with slight modification. The ABTS radical ($ABTS^+$) was generated by mixing an ABTS (7 mM) solution with a potassium persulfate (2.45 mM) aqueous solution and then leaving the mixtures in the darkness for 16 h. A total of 2.5 mL freshly prepared $ABTS^+$ solution was added to 1 mL of the plant extract, and the reaction mixture was twirled for 10 s and then the absorbance was measured at 734 nm after 6 min.

2.5.4. Determination of Fe^{2+}-chelating ability

The chelating activity of ABMP on Fe^{2+} was carried out following a reported procedure (Jin et al., 2012). ABMP of different concentrations were mixed with 3.7 mL deionized water, and then reacted with $FeSO_4$ (1.5 mM, 0.1 mL). After 0.2 mL of 5 mM ferrozine mixed in, the solution was left to stand for 10 min at room temperature, and then the absorbance of the mixture was determined at 562 nm.

Table 1
The chemical composition of ABMPs obtained by different methods.[a]

Samples	ABMP-F	ABMP-V	ABMP-A
Polysaccharide yield (%)	12.28 ± 0.30	11.54 ± 0.46	11.19 ± 0.28
Neutral sugar (%)	51.71 ± 1.27	48.92 ± 1.96	48.01 ± 1.18
Protein content (%)	3.91 ± 0.70	3.74 ± 0.16	3.75 ± 0.02
Uranic content (%)	28.19 ± 1.39	25.72 ± 1.48	24.87 ± 1.03

[a] Each value is expressed as mean ± standard deviation ($n = 3$). Means within a row are significantly different ($p < 0.05$).

2.6. Statistical analysis

All experiments were performed at least in duplicate, and analyses of all samples were operated in triplicate and averaged. Statistical analysis involved a use of the SPSS software (version 19.0; SPSS Inc., Chicago, USA). The results were presented with three determinations ± SD (standard deviation). The results were considered statistically significant if the P-values were less than 0.05.

3. Results and discussion

3.1. Yields and chemical compositions of ABMP

The yields of crude polysaccharide and chemical composition of the three ABMP samples were shown in Table 1. The yields of three crude ABMP samples were 12.28% for ABMP-F, 11.54% for ABMP-V and 11.19% for ABMP-A, respectively. The yield of ABMP-F was obviously higher than that of ABMP obtained by the other two drying methods ($p < 0.05$), which suggested that freeze drying method was a better treatment for obtaining the polysaccharide from ABM.

Some polysaccharides contain neutral sugar and uronic acid, and they are usually conjugated with other components such as protein to exhibit various performances. So it was necessary to analyze the role of neutral sugar, uronic acid and protein in ABMP samples. As shown in Table 1. ABMP-F contained a higher contents of neutral sugar (51.71%), uronic acid (28.19%) and protein (3.91%), while ABMP-A contained a lower contents of neutral sugar (48.01%), uronic acid (24.87%) and protein (3.75%). The contents of neutral sugar, uronic acid and protein evaluated in ABMP-V, ABMP-A, and ABMP-F were gradually increased from 48.01% to 51.71%, 24.87% to 28.19%, and 3.75% to 3.91%, which were correlated well with the yields. This might attribute to the destruction of the constituents in the condition of vacuum and oxygen existed (Ma et al., 2011). In previous studies, it was found that there was a direct relationship between uronic acid contents and radical scavenging effects of polysaccharides (Fan, Li, Deng, & Ai, 2012). The higher contents of uronic acid and protein might indicate stronger antioxidant ability of ABMP-F

3.2. Antioxidant activities

3.2.1. Scavenging activity on DPPH radicals

Fig. 1 illustrated the scavenging rate of all samples showing scavenging ability of ABMPs on DPPH radicals. With the extension of detecting concentration, the rates of all samples tended to ascending drastically. Among three polysaccharides, ABMP-F exhibited higher DPPH radical scavenging rate than ABMP-V and ABMP-A at

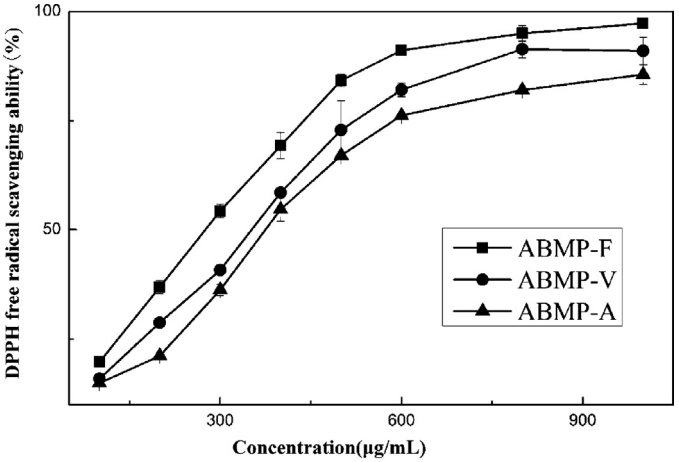

Fig. 1. Scavenging effects of ABMPs obtained by different drying methods on the DPPH radical. Results were presented as mean ± SD ($n = 3$).

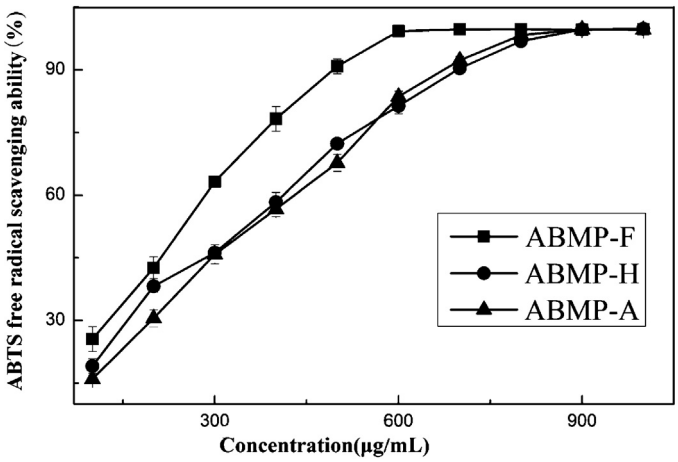

Fig. 3. Scavenging effects of ABMPs obtained by different drying methods on ABTS radical. Results were presented as mean ± SD ($n = 3$).

every concentration point, which implied that ABMP-F has the best DPPH radical scavenging ability and ABMP-A the worst. Considering the changes of the chemical composition of the three ABMP samples, it can be concluded that the DPPH radical scavenging abilities were influenced by different drying methods.

3.2.2. Scavenging activity on hydroxyl radicals

Fig. 2 showed the hydroxyl radical scavenging power of the three polysaccharides. The scavenging ability correlated well with increased concentrations of the polysaccharides. Hydroxyl radical-scavenging activity of ABMP-F and ABMP-V exhibited a relatively high level at all testing concentrations. It was important to note that the hydroxyl radical scavenging ability of ABMP-A was always lower than 50% with the concentration ranging from 0.1 to 1.0 mg/mL, indicating that it had little scavenging activity on hydroxyl radicals. ABMP-F and ABMP-V had stronger scavenging ability and higher content of uronic acid than ABMP-A. The outstanding antioxidant activities of ABMP-F and ABMP-V may be attributed to the higher content of uronic acid which can reduce the generation of hydroxyl radicals by chelating ferrous ion. Different drying methods caused the changes of uronic acid of ABMP and affected the hydroxyl radical scavenging ability (Fan et al., 2012).

3.2.3. Scavenging activity on ABTS radicals

The scavenging ability of ABMPs on ABTS free radicals was shown in Fig. 3. The scavenging power of ABMP-V and ABMP-A was slightly lower than ABMP-F at the same concentration. These results suggested that ABMP-F had the strongest scavenging power on ABTS radicals in contrast to the weakest power of ABMP-A. There was no significant difference ($P > 0.05$) between ABMP-V and ABMP-A on ABTS free radical scavenging. Moreover, ABMPs exhibited higher than 50% scavenging ABTS radical capacity when the concentration reached 0.3 mg/mL. The results indicated that ABMP had strong scavenging power on ABTS radicals and could be explored as a potential ABTS scavenger.

3.2.4. Chelating ability of polysaccharide on Fe^{2+}

The ferrous ion chelating activity of ABMP at different concentrations was shown in Fig. 4. Except for ABMP-A, and the other two polysaccharides were found to have more potent chelating ability on Fe^{2+} in a concentration-dependent manner from 0 to 1.0 mg/mL. Compared with ABMP-F in the equivalent concentration, ABMP-V and ABMP-A exhibited a much weaker metal chelating ability. Their chelating ability on Fe^{2+} decreased in the order of ABMP-F > ABMP-V > ABMP-A, so did the content of uronic acid. Several studies had demonstrated that the metal ion chelating ability of

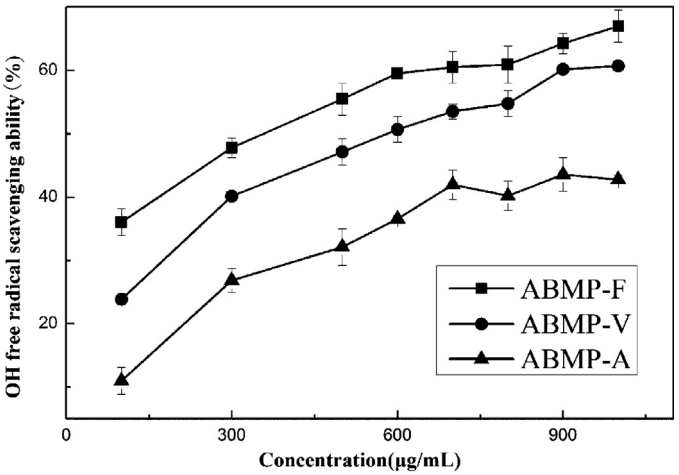

Fig. 2. Scavenging effects of ABMPs obtained by different drying methods on hydroxyl radical. Results were presented as mean ± SD ($n = 3$).

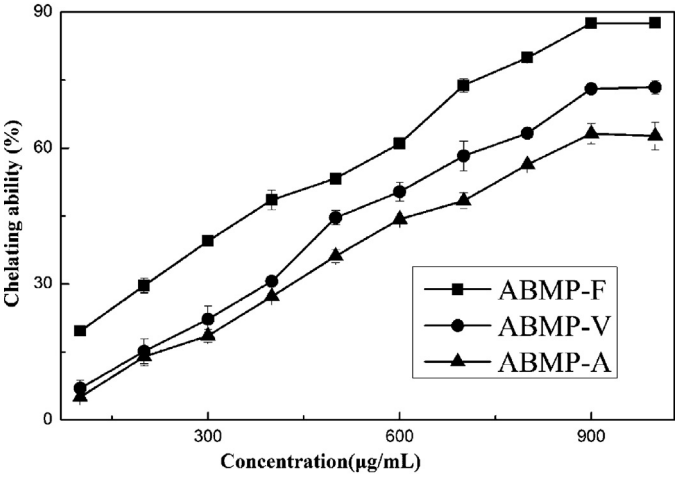

Fig. 4. Ferrous ion chelating ability of ABMPs obtained by different drying methods on Fe^{2+}. Results were presented as mean ± SD ($n = 3$).

polysaccharides could be ascribed to the formation of cross-bridge between carboxyl group in uronic acid and divalent ion.

4. Conclusion

In this study, polysaccharides were extracted from *Agaricus blazei Murrill* and treated with freeze drying, vacuum drying, air drying methods and the chemical compositions and antioxidant activities were firstly compared. ABMP-F possessed the highest yield of polysaccharides of 12.28% in contrast with the lowest yield of 11.19% of the ABMP-A among three ABM extracts. All extracts exhibited antioxidant activities in a concentration-dependent manner. The results of the present work indicated that freeze dried ABMP showed some advantages over ABMP-V and ABMP-A owing to its highest polysaccharides yield, neutral sugar content, uronic acid content, and best scavenging effects on hydroxyl radical, DPPH free radical, ABTS free radicals and strongest ferrous ion chelating ability. Furthermore, antioxidant activities studies indicated that ABMP-F and ABMP-V possessed greater capacity in scavenging free radicals, which may be related to higher content of uronic acid. The results suggested that freeze drying method was a good choice for the preparation of ABMP and was appropriate for producing antioxidants for food industry.

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