

Original Research Paper

Effects of Different Drying Methods on Antioxidant Activity of Polysaccharides from *Antler Mushroom*

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Article history

Received: 28-04-2021

Revised: 10-06-2021

Accepted: 23-06-2021

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Abstract: In this study, three polysaccharides (AMP-H, AMP-V and AMP-F) were obtained from polysaccharides of *Antler Mushroom* (AMP) by hot air drying, vacuum drying and freeze drying, respectively. The chemical composition and antioxidant capacity of AMP-H, AMP-V and AMP-F were investigated. The results showed that the AMP was an acidic polysaccharide containing a small amount of protein. The drying method did not affect the monosaccharide type, but changed the composition ratio of monosaccharide and the content of uronic acid. The content of uronic acid in AMP-H was lower significantly than that in AMP-V and AMP-F ($p < 0.05$). Antioxidant studies showed that AMP-H, AMP-V and AMP-F had strong scavenging ability against hydroxyl radical, superoxide anion radical and DPPH radical. Within a certain concentration range, antioxidant capacity of AMP-H, AMP-V and AMP-F was positively correlated with the mass concentration. The drying method had different effects on the antioxidant capacity of AMP. Compared with AMP-H and AMP-V, AMP-F had the strongest antioxidant activity. Based on the above results, freeze drying is the best drying method suitable for AMP preparation among the three tested methods.

Keywords: *Antler Mushroom* Polysaccharide, Drying Mode, Chemical Composition, Antioxidant

Introduction

Velvet *Antler mushroom* (*Lyophyllum decastes*), is a kind of rare edible mushroom with medicinal properties. It belongs to order Agaricales, dried mushroom family and *Lyophyllum* (*Arana-Gabriel et al.*, 2018). The name *Antler mushroom* is derived from mushroom shape that resembles a broom, coral and a young antler. *Antler mushroom* is rich in nutrients, including protein, vitamins, dietary fiber, eight essential amino acids, vitamins and polysaccharides. β -dextran is main functional ingredients of polysaccharides in *Antler mushroom* (AMP) containing β -1, 3-D dextran and β -1, 6-D dextran (*Zheng et al.*, 2013). Studies have shown that AMP has multiple effects (*Lee et al.*, 2011). It is a low-calorie and low-fat health product that can lower blood pressure and cholesterol and prevent senescence with antitumor, cosmetic and immunity-enhancing effects.

Antioxidant activities of polysaccharide can be affected by chemical components, molecular mass, structure, conformation and even the drying methods. Drying has become a widely used method for the preparation of polysaccharides. Various techniques

have been used in the drying process of polysaccharides. Traditional Hot air Drying (HD) is the most commonly used drying method in the food industry. However, significant quality changes of the dried product may occur during HD. Vacuum Drying (VD) is ideal for materials that can be damaged or changed by exposure to high temperatures and oxygen. Vacuum removes moisture while preventing oxidation that may occur when certain materials are combined with air (*Fan et al.*, 2012). However, drying may lead to changes in the structure of polysaccharides, thereby affecting their biological activity. How to choose a suitable drying method for polysaccharides without affecting the biological activity of polysaccharides is particularly important?

Currently, there are few reports about the effects of different drying technologies on the properties of AMP. Therefore, the purpose of this study is to explore the effects of different drying methods on the composition and antioxidant activity of AMP. On the premise of maintaining high antioxidant activity of AMP, the present study aims to find a suitable drying method for AMP and lay the foundation for the industrial application of AMP.

Materials and Methods

Materials and Equipment

Velvet *Antler mushroom* was from Shanghai Detian Agricultural Products Co., Ltd. Potassium ferricyanide ($K_3[Fe(CN)_6]$) was from Shanghai Hongrui Chemical Industry Co., Ltd. DPPH was from Shanghai Hualan Chemical Technology Co., Ltd. Hydrochloric acid, anhydrous ethanol, methanol and phenol were of analytical grade from Jiangsu Qiangsheng Chemical Co., Ltd. China.

An SL68-GJ-3 sealed sample grinder was from Zhengzhou Zhongcheng environmental protection equipment Co., Ltd. HWS-26 constant temperature water bath was from Jiangsu Jiamei Instrument Manufacturing Co., Ltd. H1750R high-speed freezing centrifuge was from Hunan Hengnuo Instrument Equipment Co., Ltd. PGC-753E precision balance was from Adam Equipment. UV-1100 visible spectrophotometer was from Jinan Siquan Medical Equipment Co., Ltd. UV1800PC ultraviolet spectrophotometer was from Tsushima Instruments, Japan. RE-2000B rotary evaporator was from Lanfan Technology Co., Ltd. SHZ-D circulating water multi-purpose vacuum pump was from Zhengzhou Zituo Instrument Equipment Co., Ltd. FDGJ-5C vacuum freeze dryer was from Changzhou Panfeng Drying Equipment Co., Ltd. 101-0A digital display electric constant temperature blast drying oven was from Jinan Zhuolong Biotechnology Co., Ltd. NicoletIS10 Fourier infrared spectrometer was from Tianjin Energy Spectrum Technology Co., Ltd. LC2010 liquid chromatograph was from Shenzhen Huadelong Technology Co., Ltd. GC-2014C Shimadzu gas chromatograph was from Shimadzu Corporation, Japan.

Preparation of AMP

The fresh *Antler mushroom* samples were rinsed with distilled water, drained and dried in a vacuum oven for 12 h at 60°C. Then, the samples were mechanically crushed through 90 mesh screens and washed two times with 95% ethanol to remove some pigments, oligosaccharides and other small molecular substances. The solvents were filtered and dried at 60°C for 24 h to obtain the *Antler mushroom* powder. The sample (100g) was placed inside a tapered bottle and then 3000mL distilled water was added. The mixture was extracted in a water bath at 75°C for 6 h and centrifuged at 3500 r/min for 30 min to obtain the extract. Then the extract was concentrated under reduced pressure to obtain the concentrate. The concentrate was deproteinized by Sevage method five times. The concentrate was purified by a polyamide adsorption column and eluted with distilled water to obtain the polysaccharide extract. Polyamide is a type of polymer compound formed by the polymerization of amide groups. Polyamide molecules are rich in amide

groups, which can form hydrogen bonds with phenols, quinones and nitro compounds to be adsorbed. Therefore, the use of polyamide as a chromatographic column filler can separate and refine certain types of substances with a certain polarity range. After the polysaccharide extract was further dialyzed for 24 h, 95% ethanol (the volume was 4 times of dialysate) was added to precipitate at 2°C for 12 h. The precipitate (AMP) was obtained by centrifugation (3500 r/min).

Drying of AMP

AMP was dried by hot air drying, vacuum drying and freeze drying to obtain hot air-dried polysaccharides (AMP-H), vacuum-dried polysaccharides (AMP-V) and freeze-dried polysaccharides (AMP-F). According to our preliminary optimization experiments, the drying parameters of the three drying methods were determined as follows: hot air-drying, 70°C with a wind speed of 2.0 m/s for 16 h; vacuum-drying, 60°C with a vacuum degree of 0.07MPa for 12 h; and freeze drying conditions, -50°C with a pressure of 0.06 MPa for 48 h. All three AMP were brownish-yellow powders with a water content of less than or equal to 5%.

Analysis of AMP Composition

Using glucose as the reference substance, the content of neutral sugar was determined by phenol-sulfuric acid method (Rasouli *et al.*, 2014). Using galacturonic acid as the standard, Glycuronate content was determined by method of m-hydroxydiphenyl (Blumenkrantz and Gasboe, 1973). Using bovine serum albumin as the standard, the Folin-phenol method was used to determine protein (Lowry *et al.*, 1951).

Analysis of the Monosaccharide Composition of AMP

The monosaccharide composition of AMP was analyzed using the derivatization of glyconitrile acetate in combination with the GC-MS (Grace *et al.*, 2013). First, 10 mg of AMP was weighed in a conical flask and added into 2 mL trifluoroacetic acid (2 mol/L). The mixture was heated in a water bath at 100°C until fully hydrolyzed. Then 10 mg sodium borohydride and an appropriate amount of inositol were mixed. The mixture was stored at room temperature for 8 h. Acetic acid was used to remove excess sodium borohydride and added into 0.5 mL acetic anhydride and 0.5 mL pyridine after decompression drying. When shaking was completed, the acetylation reaction was carried out in a constant temperature water bath at 90°C for 6h and the acetic anhydride and pyridine were dried under reduced pressure. The remaining products were dissolved in $CHCl_3$ and finally analyzed using GC-MS over a 0.22 μ m microporous membrane. 0.25 μ m \times 0.25 mm \times 30 m) was used. Gas carrier was H_2 . Flow rate was 1.5 mL/min. Injection temperature was 280°C and interface temperature was 230°C. The

temperature programmed was as follows: Starting temperature was 120°C for 3 min and then 210°C for 4 min.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis of AMP

The infrared spectrum of AMP was analyzed by KBr pressing method. First, potassium bromide was backed at 110°C in an oven for 2 h and dried. Different drying methods obtained AMP with the rate of KBr: Sample =100:1 in a dry agate bowl and mixed and pressed into transparent sheets. Scanning was performed using an FTIR spectrometer ranging from 4000 to 400 cm⁻¹ at a resolution ratio of 4 cm⁻¹ (Wang *et al.*, 2017).

Observation of AMP Morphology

First, the sample powder of AMP-F was glued to the copper sample table, the floating sample was blown off and the surface was sprayed with gold. Finally, the morphology of the sample was scanned and observed by electron microscopy.

Determination of Antioxidant Activity

Determination of DPPH Scavenging Activity

The DPPH scavenging activity was determined by adopting the reported methods (Giri *et al.*, 2011). 1 mL AMP-H, AMP-V and AMP-F (0.5, 1.0, 2.0, 3.0 and 4.0 mg/mL) were placed in a test tube and added into 2 mL DPPH-ethanol solution (0.1 mmol/L). The solution was shaken well and stored away from light for 30 min. The absorbance was determined at 517 nm using a spectrophotometer. The sample determination was performed three times. The mean value was recorded. DPPH radical scavenging rate was calculated according to the following formula:

$$\text{DPPH radical scavenging rate(\%)} = [1 - (A_1 - A_2) / A_0] \times 100$$

where A_1 is the absorbance with the presence of DPPH and tested polysaccharides. A_0 is the absorbance of the blank group without polysaccharide sample. A_2 is the absorbance of the control group with the presence of tested polysaccharides but without DPPH.

Determination of Superoxide Anion Scavenging Activity

The superoxide anion radical scavenging effect was determined using the reported method (Jiang *et al.*, 2010) with slight modifications. 4.5 mL Tris-HCl buffer solution (50 mmol/L, pH 8.2), 1 mL AMP-H, AMP-V and AMP-F solutions (0.5, 1.0, 2.0, 3.0 and 4.0 mg/mL) and 0.4 mL pyrogallol acid (3.0 mmol/L) were mixed and stood at 25°C for 20 min. Then the mixed solutions were heated

for 5 min at 25°C. Thereafter, 0.5 mL thick hydrochloric acid was added to terminate the reaction. Absorbance was determined at 299 nm and recorded as A_1 . The blank control group was replaced by the polysaccharide sample solution with Tris-HCl buffer. The sample determination was repeated three times. The mean value was recorded. Superoxide anion radical scavenging rate was calculated according to the following formula:

$$\text{Superoxide anion scavenging rate(\%)} \\ = (A_0 - A_1) / A_0 \times 100$$

where A_0 is the absorbance value of Tris-HCl buffer instead of polysaccharide sample. A_1 is the absorbance value of polysaccharide sample at various concentrations.

Determination of Hydroxyl Radical Scavenging Activity

1 mL AMP-H, AMP-V and AMP-F solutions (0.5, 1.0, 2.0, 3.0 and 4.0 mg/mL), 1 mL FeSO₄ solution (9.0 mmol/L) and 1 mL salicylic acid ethanol solution (9.0 mmol/L) were mixed. 1.0 mL H₂O₂ (8.8 mmol/L) were added into the mixture to start the reaction. The mixed reaction solution was incubated at 37°C for 30 min. Absorbance at 562nm was determined as A_1 . Distilled water instead of polysaccharide sample solution was recorded at 562 nm, which was recorded as A_0 . In the blank control group, distilled water was used instead of salicylic acid ethanol solution. The absorbance at 562 nm was measured and recorded as A_2 . The measurement was performed three times. The hydroxyl radical scavenging rate was calculated as follows:

$$\text{Hydroxyl radical scavenging rate(\%)} = [1 - (A_1 - A_2) / A_0] \times 100$$

where A_1 is the absorbance of polysaccharide sample. A_2 is the absorbance without salicylic acid-ethanol solution. A_0 is the absorbance of the control (distilled water instead of the polysaccharides).

Results and Discussion

Chemical Constituents of AMP

Extracted polysaccharides from natural products often have neutral sugars and glyoxylic acid. Covalent bonds with proteins form proteoglycans with biological functions (Ma *et al.*, 2013). Neutral sugars, glyoxylic acid and proteins were measured in AMP-H, AMP-V and AMP-F as shown in Table 1. Acidic polysaccharides, referred to as polysaccharides containing acidic groups, mainly include glyoxylic acid, sulfuric acid group and phosphoric acid group. Their chemical structures may also contain neutral sugar. Therefore, the three polysaccharides are all acidic polysaccharides containing a small amount of protein (Yan *et al.*, 2019). The content of neutral sugar and

protein in the three polysaccharides was low. The content of uronic acid was 4.09, 4.91 and 4.84% in AMP-H, AMP-V and AMP-F, respectively. The content of uronic acid in AMP-V and AMP-F was significantly higher than that in AMP-H ($p < 0.05$). The possible reason is that structure of the uronic acid in AMP-H is destroyed under dry, oxygen and high temperature conditions (Chen and Huang, 2019). In this experiment, the uronic acid content of AMP-H, AMP-V and AMP-F was less than 5%. The reason may be that neutral sugars and uronic acid interacted, not both independent. They may exist in a sugar chain and cannot be separated using existed methods. On the other hand, the content of neutral sugar and uronic acid is determined by different methods, which will interfere with each other.

Monosaccharide Composition of AMP

AMP monosaccharide composition was analyzed by GC-MS technique. Table 2 showed that the monosaccharide compositions of the three AMP were galactose, ribose, mannose, fucose, rhamnose, glucose and xylose, but its molar ratio had changed. The monosaccharide molar ratios of AMP-H, AMP-V and AMP-F were 1:13.63:50.62:34.38:284.08:80.36, 1:5.24:69.09:45.26:300.31:70.41 and 1:6.82:73.18:25.71:332.05:63.75, respectively. The results showed that the drying method generally did not change the monosaccharide species of polysaccharides but affected their composition ratios, which may be due to the change in monosaccharide conformation during drying (Wang *et al.*, 2013). Besides, different drying methods can directly affect the molecular weight and spatial composition of polysaccharides (Wu, 2015).

Fourier Transform Infrared Spectroscopy Analysis of AMP

According to the positions and characteristics of the characteristic peaks in the infrared spectrum, the structural characteristics of polysaccharides can be inferred. Therefore, the basic method which can qualitatively identify the structure of polysaccharides is mainly infrared spectroscopy. Figure 1 showed that the infrared spectra of AMP-H, AMP-V and AMP-F was the same. In the range of $4000-400\text{ cm}^{-1}$, AMP-H, AMP-V and AMP-F had the general characteristics of polysaccharides. AMP-H, AMP-V and AMP-F showed strong peaks at 3400, 2900, 1600 and 1400 cm^{-1} . The strong absorption peak near 3400 cm^{-1} is mainly attributed to O-H and N-H stretching vibrations. The absorption peak at 2900 cm^{-1} is attributed to the stretching vibration of the C-H bond. The absorption peaks at 1600 and 1400 cm^{-1} are attributable to the stretching vibration of the C-H bond and the deformation characteristics of $=\text{CH}_2$. The absorption peaks around 1600 and 1400 cm^{-1} indicated the presence of carboxylic acids in polysaccharides (Liu *et al.*, 2017). Therefore, AMP-H, AMP-V and AMP-F were acid polysaccharides. The absorption peak at 600 cm^{-1} was the skeleton symmetric stretching vibration absorption

peak of pyranose (Černá *et al.*, 2003). Therefore, AMP is a kind of pyranose.

Electron Microscope Analysis

Figure 2 showed the AMP morphology under a scanning electron microscope. At $2500\times$ magnification, the polysaccharide samples were mostly reticular, indicating that the polysaccharide had a high molecular weight, long-chain or branched structure and ability to cross adhere between molecules easily. After 5000 times of exposure, the surface of the polysaccharide samples showed a smooth and flat sheet structure, indicating the interaction between polysaccharides and their ability to form tight structures easily (Shang *et al.*, 2013).

Antioxidant Effect of AMP in Vitro

Scavenging Effect of AMP-H, AMP-V and AMP-F on DPPH Free Radicals

The DPPH assay was used to assess antioxidant activity of extracted polysaccharides. DPPH is a relatively stable free radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule, which has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants (Ke *et al.*, 2015). The scavenging effects of polysaccharides named GLP-F, GLP-V and GLP-H on the DPPH radical were in a concentration-dependent fashion (Fan *et al.*, 2012).

Figure 3 showed that the AMP obtained by hot air drying, freeze drying and vacuum drying have a good ability of scavenging DPPH free radicals. The scavenging effect was positively correlated with the mass concentration. As the mass concentration of AMP-H, AMP-V and AMP-F increased, the scavenging effect also increased. The scavenging effect of AMP-F, AMP-V and AMP-H on DPPH free radicals weakened in turn. When the polysaccharide concentration reached 4.0 mg/mL , DPPH free radical scavenging rate of AMP-H, AMP-V and AMP-F was 58.9, 65.0 and 89.1%, respectively. The DPPH radical scavenging abilities were influenced by the different drying method because of the changes of the physicochemical properties of polysaccharide samples (Ma *et al.*, 2013).

Scavenging Effect of AMP-H, AMP-V and AMP-F on Superoxide Anion Radicals

Superoxide anion free radicals can accelerate the aging process from the skin to the internal organs (Lagercrantz, 1999). Accordingly, it can induce many diseases by damaging important biological macromolecules (Sun *et al.*, 2009). Figure 4 showed that AMP obtained by hot air drying, freeze drying and vacuum drying can effectively scavenge superoxide anion free radicals. The scavenging effect of the three AMP polysaccharides on superoxide anion radical increased when concentration increased.

AMP-F had the strongest scavenging ability, whereas AMP-H had the weakest. When the polysaccharide concentration reached 4.0 mg/mL, the scavenging rate of AMP-H, AMP-V and AMP-F on superoxide anion radical was 59.5, 62.2 and 88.3%, respectively.

Scavenging Effect of AMP-H, AMP-V and AMP-F on Hydroxyl Radicals

Hydroxyl radical is considered to be a strong oxidant, which can interact with most biological macromolecules and induce serious damage to the adjacent biomolecules (Fan *et al.*, 2012). Therefore,

scavenging hydroxyl free radicals is important for the antioxidant defense in the cell or food system.

Figure 5 showed that AMP dried by hot air drying, freeze drying and vacuum drying can scavenge hydroxyl radical as well. With the increase of the concentration of AMP-H, AMP-V and AMP-F, the scavenging effect of hydroxyl radicals increased within a certain concentration range. When the concentration was 4 mg/mL, the scavenging effect of AMP-H, AMP-V and AMP-F on hydroxyl free radicals is the highest, respectively. Among them, the scavenging effect of AMP-F on hydroxyl free radicals is higher than that of AMP-H and AMP-V.

Table 1: Effects of different drying methods on the chemical composition of AMP

| Sample | AMP-H | AMP-V | AMP-F |
|-------------------|-------------|-------------|-------------|
| Neutral sugar (%) | 85.20±1.75a | 85.16±1.12a | 86.23±1.62a |
| Protein (%) | 2.26±0.12a | 2.38±0.17a | 2.34±0.10a |
| Uronic acid (%) | 4.09±0.25b | 4.91±0.31a | 4.84±0.27a |

Note: The data in the table are the average ± standard deviation of the data repeated 3 times. The data corresponding to the same peer with different lowercase letters is significant ($p < 0.05$)

Table 2: Effects of different drying methods on AMP monosaccharide composition

| Sample | AMP-H | AMP-V | AMP-F |
|---------------|--------------|--------------|--------------|
| Xylose (%) | 41.69±0.19b | 43.00±0.22b | 49.27±0.91a |
| Glucose (%) | 28.84±0.32a | 25.67±0.29b | 23.60±0.50c |
| Galactose (%) | 1.21±0.01c | 2.73±0.04a | 2.65±0.13b |
| Mannose (%) | 3.13±0.57a | 2.42±0.35b | 2.41±0.09b |
| Rhamnose (%) | 16.89±0.88b | 20.02±0.34a | 10.99±0.18c |
| Ribose (%) | 0.21±0.0004c | 0.45±0.0038b | 0.90±0.0030a |
| Fucose (%) | 11.31±0.63c | 17.24±0.18b | 23.07±0.45a |

Note: The data in the table are the average ± standard deviation of the data repeated 3 times. The data corresponding to the same peer with different lowercase letters is significant ($p < 0.05$)

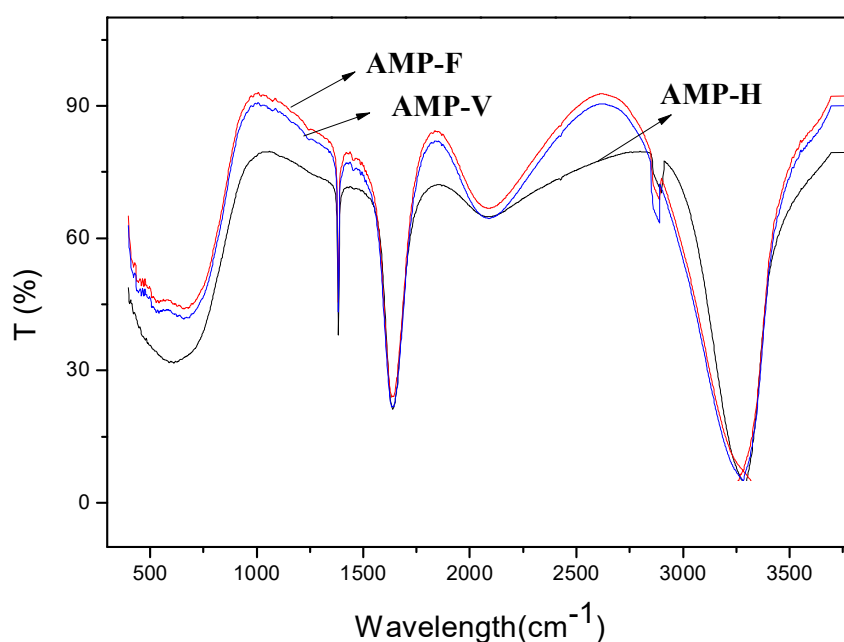


Fig. 1: Infrared spectra of AMP dried by different methods

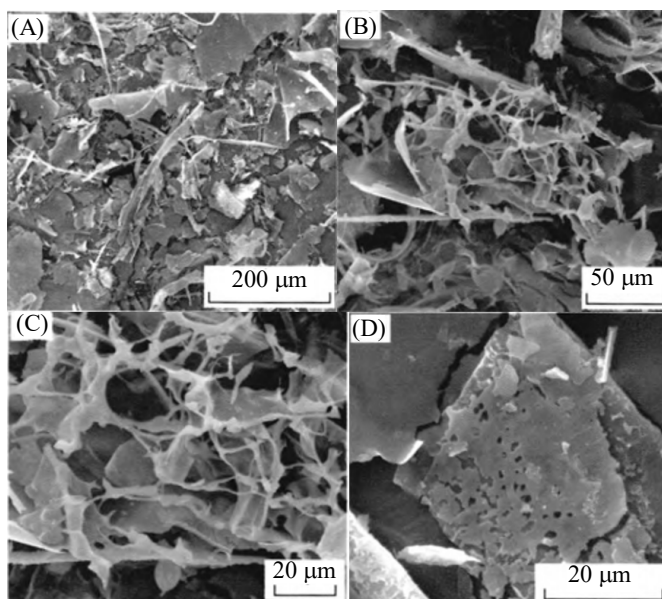


Fig. 2: Observation of AMP under electron microscope with different magnification at 500(A), 1300(B), 2500(C) and 5000(D)

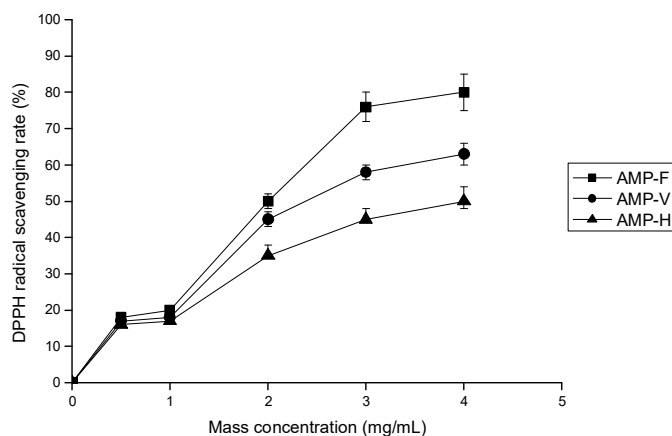


Fig. 3: Scavenging effect of AMP dried by different methods on DPPH free radicals

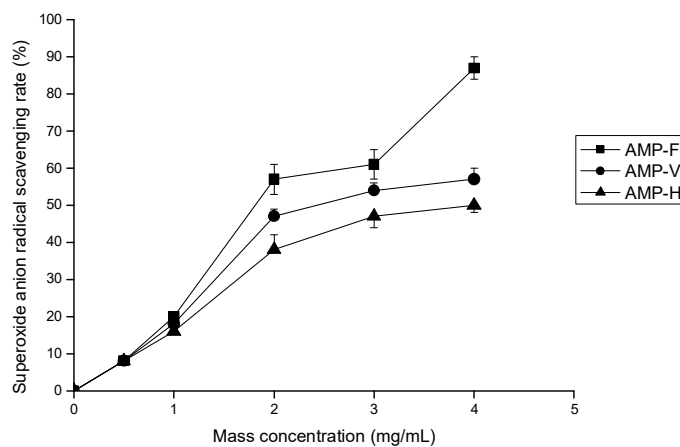


Fig. 4: Scavenging effect of AMP dried by different methods on superoxide anion free radicals

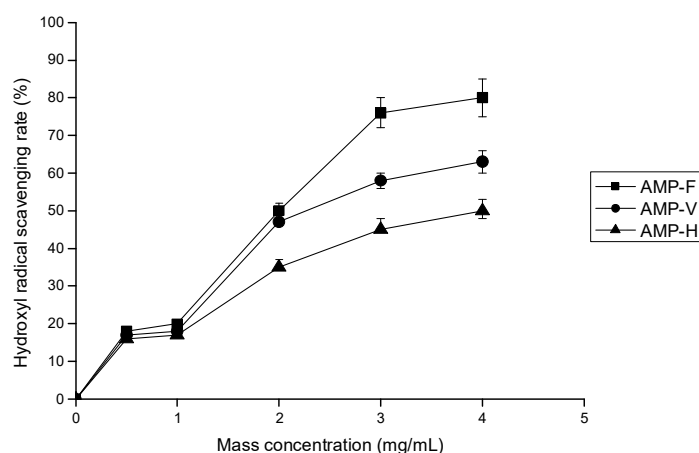


Fig. 5: Scavenging effect of AMP dried by different methods on hydroxyl radicals

Conclusion

In this study, hot air drying, freeze-drying and vacuum-drying were used to dry AMP to obtain AMP-H, AMP-V and AMP-F, respectively. The chemical composition and antioxidant capacity of AMP-H, AMP-V and AMP-F were investigated. AMP-H, AMP-V and AMP-F were all acidic polysaccharides containing a small amount of protein. The monosaccharide species of AMP-H, AMP-V and AMP-F were unchanged, whereas their monosaccharide compositions were different. The content of uronic acid in AMP-H was lower significantly than that in AMP-V and AMP-F ($p < 0.05$). The infrared spectrum analysis showed that the chemical structure of AMP-H, AMP-V and AMP-F was basically the same. The antioxidant activity in vitro showed that drying mode had a significant effect on the antioxidant ability of AMP. Compared with AMP-V and AMP-H, AMP-F had the strongest antioxidant activity. Therefore, freeze drying is the best drying method for preparing AMP. This work provides a theoretical basis for the development and utilization of AMP.

Acknowledgement

This research was supported by Suzhou Science and Technology Plan Project (SNG2020064).

Author's Contributions

Bing Xu and Yi Dai: Participated in all experiments and article writing.

Shiyu Xu, Jianjun Liu and Botao Hu: Participated in drying of AMP and data processing.

Xiao Ding and Xinyi Wu: Participated in antioxidant activities of AMP-H, AMP-V and AMP-F.

Yiyong Chen: Participated in project design and experimental guidance of this manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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