A Comparative Study of Polysaccharides in Fruiting Bodies and Environmentally Friendly Fermentation Broth for Ecological Security Protection

Hailong Yu, Shuangshi Li, Bin Jiang, Zheng Yan*, Hui Liu
College of Bioengineering, Beijing Polytechnic, Beijing 100176, China

*Corresponding author: yan_zheng@bgy.edu.cn

Abstract. To provide theoretical and technical support for the broad application of fermentation polysaccharides by Fomes officinalis, the differences of polysaccharides extracted from fruiting bodies and fermentation broth of F. officinalis Ames were compared. Polysaccharide concentration was determined by the phonel-sulfate method, and monosaccharide compositions were analyzed by ion chromatography-integrated pulsed amperometric detector. Also, the antioxidant activities of the two samples were determined based on DPPH and hydroxyl radical scavenging oxidation experiments. The results indicated that polysaccharide contents of fruiting bodies and fermentation broth were 71.23% and 67.12%, respectively. The monosaccharide composition of fruiting body polysaccharide was glucose, galactose, mannose, and xylose from more to less, and that of fermentation polysaccharide was glucose, mannose, galactose, xylose, and arabinose. Both fruiting polysaccharide and fermentation polysaccharide are mainly composed of macromolecular polysaccharide, and the scavenging ability of hydroxyl radical is similar. The scavenging ability of DPPH radical of fermenting polysaccharides is higher than that of fruiting polysaccharides. Although there were some differences in polysaccharide contents, monosaccharide composition and molecular weight distribution between fruiting bodies and fermentation broth, the antioxidant activity of the two samples was similar.

Keywords: Fomes officinalis Ames, fruiting bodies, fermentation broth, polysaccharides

1. Introduction
Fomes officinalis Ames (Alihong in Chinese) is a rare medicinal fungus of Fomes genus in Polyporaceae family [1]. Uyghur doctors believe that the dried fruit body of F. officinalis Ames has many functions such as warming lung, eliminating phlegm, reducing asthma, enhancing physical strength, prolonging the anti-fatigue and hypoxia tolerance time. It is often used as a health care drug to improve the emergency response capacity of the body, and can also be washed and drunk to enhance human immunity [2]. Modern scientific research shows that polysaccharides, which are the main active components of F. officinalis Ames, are capable of eliminating free radicals, anti-aging, immune regulation, antibacterial and antiviral effects [3]. At present, the source of F. officinalis Ames mainly depends on the field picking, with the excessive exploitation of resources, the natural resources of F. officinalis Ames are facing severe pressure.
Ames are gradually reduced, which is challenging to meet the growing demand. With the development of fermentation engineering technology, the preparation of \textit{F. officinalis} Ames polysaccharide by liquid fermentation has attracted more and more attention [4]. This method has the advantages of low raw material cost, short production cycle, stable product quality and easy process control. To obtain the similarities and differences between the two kinds of polysaccharides from various sources, two polysaccharides from \textit{F. officinalis} Ames (Fer-P from the fermentation broth and Fru-P from the fruiting body) were obtained, and the structural characterizations, antioxidant activities of them were investigated.

2. Materials and Methods

2.1. Materials and reagents

The dried fruiting bodies of \textit{F. officinalis} Ames, were collected from Yanbian Huaxia Sanghuang fungus industry Co., Ltd (Jilin, China). \textit{F. officinalis} Ames (ACCC 50561) was purchased from Agricultural Culture Collection of China, and maintained on potato dextrose agar (PDA) slant at 4°C and transferred every two months. 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH•) and D-glucose were purchased from Sigma-Aldrin Chemical Co. (St. Louis, MO). Ascorbic acid (Vc) was purchased from Sinopharm Chemical Reagent Co. (Beijing, China). All other chemicals were of analytical grade.

2.2. Medium

Slant medium [5]: potato dextrose agar (PDA). Liquid seed medium (L): glucose 30.0 g, peptone 15.0 g, KH₂PO₄ 3.0 g, MgSO₄·7H₂O 1.0 g, VB1 0.01 g, pH value natural. Liquid fermentation medium (L): glucose 10.0 g, malt extract 3.0 g, peptone 7.5 g, yeast extract 5.0 g, pH value natural.

2.3. Extraction of polysaccharide from the fruit body

The dried fruiting bodies of \textit{F. officinalis} Ames were crushed into fine powders using a disintegrator (BJ-150, Deqing Baijie Electric Co., Zhejiang, China) and sieved (40 mesh). The dry powder was extracted twice by hot water at 100°C in a 1:20 (w/v) ratio for 2.5 h. The aqueous extracts were filtered and concentrated, then precipitated with four volumes of 95% ethanol for overnight at 4°C. The precipitated polysaccharide was obtained by centrifuging at 10,000 r/min for 10 min and redissolving with an appropriate amount of distilled water. The aqueous solution was dialyzed (cut off Mw 3500 Da) with distilled water for two days to yield the Fru-P.

2.4. Preparation of fermentation polysaccharide

2.4.1. Inoculum preparation, flask culture and bioreactor fermentation

The pre-cultivation, inoculated from slants, was grown in 500 mL shake flasks containing 150 mL pre-culture medium at 28°C and 160 rpm for 3 d. The bioreactor fermentation was performed in a 5 L bioreactor (BIOTECH-5BG-2, Shanghai Baoxing Bio-engineering Co., China) with an initial volume of 3 L at 28°C, and the agitation speed was set at 200 r/min.

2.4.2. Extraction of fermentation polysaccharide

The fermentation broth was centrifuged at 10,00 r/min for 10 min to remove the mycelium, and then the supernatant was concentrated, alcohol precipitated and centrifuged to collect polysaccharide. After freeze-drying, the polysaccharide was obtained from the fermentation broth of \textit{F. officinalis} Ames (Fer-P).

2.4.3. Determination of polysaccharide content in crude polysaccharide

Take 0.5 g of the crude polysaccharides from Fru-P and Fer-P, and then add water to reconstitute to 5 mL. The concentration of polysaccharide was determined using the phenol-sulfuric acid method [6], and then the polysaccharide content (C) in the crude polysaccharide is calculated according to formula (1).
\[ C(\%) = \frac{m \times V}{M_0} \times 100\% \]  

\( m \): the polysaccharide concentration, \( V \): the Volume of the sample solution, \( M_0 \): the dry weight of crude polysaccharide. The experiment was repeated three times, each with duplicate samples.

### 2.5. Determination of monosaccharide composition

The monosaccharide composition of polysaccharides from Fru-P and Fer-P were analyzed by ion exchange chromatography. The specific operation was as follows [7, 8]:

- **Hydrolysis**: 5.0 mg of polysaccharide sample was added into 10 mL ampere tube, and 2 mL of 2.0 mol/L trifluoroacetic acid solution was added, and then the tube was filled in nitrogen for 5 min to prevent the sample from oxidation. After sealing the pipe orifice, hydrolyzing was taken at 120°C for 2 h. And then 5.0 mL methanol was added into the tube after cooling to room temperature. Finally, 1.0 mL of water was added into the ampere tube to obtain the hydrolysate of polysaccharide sample. The solution was diluted and filtered with 0.22 μM filter membrane for standby.

- **Detection**: ion chromatography protection column (Carbo PacTMPA20 BioLCTM, 3 mm×30 mm), chromatographic column (Carbo PacTMPA20, 3 mm×150 mm), Detector: pulse amperometric detector, mobile phase: A: ultra pure water, B: 250 mmol/L NaOH solution, flow rate: 0.5 ml/min, injection volume: 20 μL. Monosaccharide standards include rhamnose, arabinose, galactose, glucose, mannose and xylose.

### 2.6. Antioxidant assays

#### 2.6.1. DPPH• assay.

The radical scavenging activity of different extracts was determined by using DPPH assay according to Jing et al. [9] after some minor modifications. Each test compound at final concentrations in the reaction ranging from 0.2 to 1.0 mg/mL was added to 1.0 mL of a DPPH• solution (0.4 mmol/L in ethanol). And Vc was used as a positive control. The sample absorbance was read at 517 nm after a 30 min incubation at room temperature in the dark. The DPPH• scavenging activity (E) was calculated according to formula (2):

\[ E(\%) = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] \times 100\% \]  

\( A_1 \): the DPPH• concentration with test compound, \( A_0 \): the DPPH• concentration without test compound, \( A_2 \): the test compound without DPPH•. The inhibition curves together with IC_{50} values were determined.

#### 2.6.2. Hydroxyl radical (HO•) scavenging assay.

The radical scavenging activity of different extracts was determined by using HO• scavenging ability according to Jing et al. [9] after some minor modifications. The reaction mixture contained 1.0 mL of FeSO₄ (5.0 mmol/L), 1.0 mL of H₂O₂ (5 mmol/L), 1.0 mL of sodium salicylate (5.0 mmol/L), and 1 mL of different polysaccharide samples. Deionized water and Vc were used as the blank and positive control, respectively. After 30 min incubation at 25°C, absorbance at \( \lambda =510 \) nm was determined. The HO• scavenging activity (E) was calculated according to formula (3):

\[ E(\%) = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] \times 100\% \]  

\( A_0 \): the presence of the deionized water, \( A_1 \): the presence of the test sample or Vc, \( A_2 \): the presence of reagent blank without sodium salicylate. The inhibition curves together with IC_{50} values were determined.
3. Results

3.1. Polysaccharide content
The polysaccharide content of Fru-P and Fer-P by phenol sulfuric acid method are shown in Figure 1. It can be seen from Figure 1 that the polysaccharide contents of Fru-P and Fer-P are similar, which are 71.23% and 67.12% respectively, indicating that polysaccharide is the primary water-soluble component in the fruit body and fermentation broth of F. officinalis Ames, and the polysaccharide content of Fru-P is slightly higher than that of Fer-P.

![Figure 1. Polysaccharide content from samples of Fru-P and Fer-P](image)

3.2. Monosaccharide composition
The monosaccharide composition of Fru-P and Fer-P detected by ion chromatography method are shown in Table 1. It can be seen from table 1 that Fru-P was mainly composed of glucose, galactose, mannose, and xylose in descending order, while Fer-P was mainly composed of glucose, mannose, galactose, xylose, and arabinose. Although the monosaccharide composition of Fru-P and Fer-P were quite different, the content of glucose was the highest in both polysaccharides.

| Sample | Monosaccharide composition (%) |
|--------|--------------------------------|
|        | Rhamnose | Arabinose | Galactose | Glucose | Mannose | Xylose |
| Fru-P  | —        | —         | 17.13     | 71.28   | 5.90    | 5.69   |
| Fer-P  | —        | 0.17      | 1.18      | 93.04   | 5.01    | 0.60   |

3.3. Antioxidant activities

3.3.1. *DPPH*• scavenging activity. The results of *DPPH*• scavenging ability of Fru-P and Fer-P are shown in Figure 2. It can be seen from Figure 2 that the scavenging ability of Fru-P and Fer-P to *DPPH*• increases with the increase of sample concentration. Although the scavenging capacity of Fru-P and Fer-P is relatively lower than that of VC, both Fru-P and Fer-P have significant *DPPH*• scavenging capacity. Although the polysaccharide content of Fru-P is higher than that of Fer-P, the *DPPH*• scavenging ability of Fer-P is more vital than that of Fru-P at the same sample concentration, which indicates that the content of polysaccharide with *DPPH*• scavenging activity in Fer-P is higher than that of Fru-P, and the relationship between polysaccharide content and antioxidant capacity is not a simple dose effect relationship. The *DPPH*• scavenging rates of Fru-P and Fer-P were 83.4% and 71.8% respectively when the sample concentration was 5.0 mg/mL.
3.3.2. Determination of hydroxyl radical scavenging capacity. The results of scavenging ability of Fru-P and Fer-P on hydroxyl radical are shown in Figure 3. It can be seen from Figure 3 that the scavenging ability of Fru-P and Fer-P on hydroxyl radical is similar to that on DPPH•, both of which increase with the increase of sample concentration. At the same sample concentration, the scavenging ability of Fru-P and Fer-P on hydroxyl radical was similar. The hydroxyl radical scavenging rates of Fru-P and Fer-P were 75.8% and 79.1% respectively when the sample concentration was 5.0 mg/mL.

Figure 2. Scavenging effects of Fru-P and Fer-P on DPPH free radicals

Figure 3. Scavenging effects of FRU-P and FER-P on hydroxyl free radicals

4. Discussion
As one of the main active components of *F. officinalis* Ames [10], polysaccharides are closely related to a variety of biological activities. Since the relationship between the composition and structure of polysaccharides and their efficacy and activity is not very clear, the content of polysaccharides has been used as one of the main indicators to measure the quality of samples.

The monosaccharide composition of Fru-P and Fer-P was not identical. Fungal polysaccharides are usually heteropolysaccharides, and their monosaccharide composition may be closely related to the nutritional source of fungi. The accumulation of polysaccharides in fungal fruiting bodies is mainly associated with plant growth environment, sampling season and other factors [11]. In contrast, the fermentation polysaccharide is mainly related to fermentation conditions, bacteria and other factors, which may be the main reason for the difference in polysaccharide content between Fru-P and Fer-P. Yu et al. [12] found that the monosaccharide composition of *Ganoderma lucidum* fruiting body and mycelium polysaccharide was significantly different, Yang [13] found that the monosaccharide composition of *Grifola frondosa* fruiting body, mycelium and fermentation broth was also different. The fruiting bodies of *F. officinalis* Ames were collected from the stems of *Larix gmelinii*. Cellulose, hemicellulose and lignin in plant fiber were the primary nutrient sources. Glucose was the main carbon source in *F. officinalis* Ames fermentation medium, which may be one reason why the proportion of
glucose in the monosaccharide composition of Fer-P was higher than that of Fru-P. Black et al. [14] analyzed the monosaccharide composition of polysaccharides by gas chromatography/mass spectrometry, and found that the monosaccharide components of polysaccharides were mannose, arabinose and galactose, which were different from the experimental results in this study. On the one hand, it may be caused by different analysis methods, on the other hand, different production areas and different analytical purification methods.

Liquid fermentation method is expected to be the main method for obtaining stable and high-quality _F. officinalis_ Ames polysaccharides due to its obvious advantages [15]. At present, it is challenging to get polysaccharides with uniform molecular weight by current separation methods. It is usually a mixture of multiple polysaccharides obtained from nature. The molecular weights of active polysaccharides reported vary from thousands to millions of daltons. Up to now, the molecular weight range of the active polysaccharide of _F. officinalis_ Ames is still lack in-depth study. The experimental results in this paper show that although the main components of Fru-P and Fer-P are macromolecular polysaccharides, the peak shape of molecular weight distribution is quite different, and the relationship between molecular weight and the efficacy and activity of _F. officinalis_ Ames polysaccharide needs further study.

Polysaccharide is a kind of biological macromolecular compound with a variety of physical activities. Antioxidant activity is a common method for screening active polysaccharides and studying the efficacy and action of polysaccharides. The results showed that Fru-P and Fer-P had significant antioxidant activity in vitro. The DPPH• scavenging ability of Fru-P was higher than that of Fer-P, but the hydroxyl radical scavenging ability of Fru-P was similar. The antioxidant activity of polysaccharides is mainly related to the -OH and -O- functional groups in monosaccharide molecules such as glucose [16-21], and the purity, monosaccharide composition and molecular weight.

5. Conclusions
The results showed some differences in polysaccharide content, monosaccharide composition, molecular weight distribution and antioxidant activity between Fru-P and Fer-P. Although the polysaccharide content of Fru-P was higher than that of Fer-P, their hydroxyl radical scavenging ability was similar, and the DPPH• scavenging ability of _F. officinalis_ Ames fermentation polysaccharide was higher than that of _F. officinalis_ Ames Fruit body polysaccharide. With the development of modern biotechnology and the in-depth study on the structure-activity relationship of active polysaccharides, fermentation technology has become one of the main methods for directional active fungal polysaccharides due to its advantages of low cost, short cycle, effortless control, large yield and stable quality.

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