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# Chemical and rheological properties of polysaccharides from fruit body of *Auricularia auricular-judae*

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ABSTRACT

Polysaccharide was extracted from *Auricularia auricular-judae* with hot water (AP). AP consisted mostly of carbohydrates (72%) and proteins (8%). The monosaccharide compositions mostly are Glucose (62%), Mannose (33%) and a small amount of Galactose (5%). The extracellular polysaccharide dispersions showed shear-thinning (pseudoplastic) behavior and its pseudoplasticity was more pronounced for 2% polysaccharide dispersion. The Power-law model was used to evaluate the viscosity curves of AP and both its viscosity and consistency indices changed as the concentration increased. The viscosity of polysaccharides dispersion decreased with the addition of salt and also at extreme pH values. AP dispersion behaved as a weak gel in the concentration of 0.5% and 1% and as a true gel at higher concentration (e.g, 2%). The addition of 1 M $CaCl_2$ to AP and changing dispersion pH to acid or alkali decrease its gel strength. AP exhibited excellent thermal stability as assessed by rheology and DSC, suggesting the material could be used in food system which requires heat tolerance.



## 1. Introduction

Polysaccharides have been used as thickeners, stabilizers, gelling agents and binding agents in food, pharmaceutical and cosmetic industry for hundreds, or in some cases, thousands of years (Alves et al., 2010). Recently, there is increasing demand for hydrocolloids with specific functions, researchers to find new sources of polysaccharides with potential industrial applications has become important (Shao, Qin, Han, & Sun, 2014; Yadav, Johnston, Hotchkiss Jr, & Hicks, 2007). In addition to the physicochemical characteristics of polysaccharides, detailed knowledge of the rheological properties of natural material is also crucial for developing its applications (Nickerson, Paulson, & Speers, 2004).

*Auricularia auricular-judae*, also called jelly ear or wood ear, is one of the most popular mushrooms in China, Korea, Japan and Viet Nam. It has been used as edible and medicinal mushroom for hundreds of years (Nguyen et al., 2012; Sone, Kakuta, & Misaki, 1978). The fruit body of *A. auricular-judae* could be easy distinguished by its noticeably ear-like shape and brown colouration. As an important active component, polysaccharides from *A. auricular-judae* have been reported to possess various bioactivities. For example, a β-D-glucan from *A. auricular-judae* exhibited potent anti-tumor activity by its inhibition against Acinar cell carcinoma proliferation and could induce apoptosis in S-180 tumor cell by up-regulating Bax and down-regulating Bcl-2 (Ma, Wang, Zhang, Zhang, & Ding, 2010). Yuan suggested that the water-soluble polysaccharides from fruiting bodies of *A. auricular-judea* had a hypoglycemic effect on non-insulin-dependent diabetes mice (Yuan, He, Cui, & Takeuchi, 1998). Water extracted polysaccharides from *A. auricular-judae* mycelium significantly decreased the levels of total cholesterol and triglyceride in mice in which hyperlipidemia had been induce (Zeng et al., 2013). Wu reported that *A. auricular-judae* polysaccharides treatment could improve heart function through its strong antioxidant activity (Wu et al., 2010). Besides, an acidic polysaccharide showed highly anticoagulant activity and obvious inhibitory effect on platelet aggregation (Yoon et al., 2003). Since the structure of polysaccharides is important for understanding their bioactivities, the chemical composition and chain conformation of polysaccharides from *A. auricular-judae* were also extensively investigated. The water-soluble β-D-glucans from fruit body of *A. auricular-judae* mainly consisted of a backbone

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chain with (1 → 6)-glucose residues (Misaki, 1981; Sone et al., 1978; Xu, Xu, & Zhang, 2012; Zhang, Yang, Ding, & Chen, 1995) while water-insoluble polysaccharides from that was a β-(1 → 3)-D-glucan with extremely branched structure (Sone et al., 1978; Zhang et al., 1995). The water-soluble neutral polysaccharides extracted from *A. auricular-judae* exhibited extended stiff chain conformation; on the contrary, the water insoluble polysaccharides isolated from that by alkali appeared have a flexible chain in aqueous solutions (Xu et al., 2012; Zhang et al., 1995). Though the structure and the bioactivities of polysaccharides from *A. auricular-judae* have been extensively studied, to the best of our knowledge, the information on the rheological properties of these polysaccharides was rare. Xu reported neutral polysaccharides from *A. auricular-judae* exhibited gel-like behavior and thermal stability, however, systematic evaluation would be required to gain a deeper insight into the rheological properties of the *A. auricular-judae* polysaccharides (Xu et al., 2012).

Therefore, the objectives of this work were to identify the chemical component of the polysaccharides separated from *A. auricular-judae*, and investigate the effects of concentration, temperature, pH and salt concentration on the rheology of this polysaccharide, which might provide useful information on the potential utilization of this new hydrocolloid source in the food industry.

## 2. Material and method

### 2.1. Materials

The fruiting bodies of *A. auricular-judae*, commercial product cultivated in Heilongjiang province (China), were washed with distilled water and air-dried at 60 °C in a drying oven (DX400, Yamato Scientific Co., Ltd., Tokyo, Japan). The dried sample was milled using a blender (Waring Products Corp., New York, N. Y., USA), sieved (40-mesh) and then stored at 4 °C before analyses. All chemicals and reagents used in this work were of analytical grade.

### 2.2. Extraction of the crude polysaccharide

The powdered sample (20 g) was refluxed with 80% ethanol (EtOH, 400 ml) at 60 °C under constant mechanical stirring for 2 h to remove the lipophilic substances and other low-molecular-weight compounds, before being centrifuged at 5000 g for 10 min and dried at 25 °C. The dried biomass was extracted in boiling water (1000 ml) for 2 h with constant mechanical stirring. The extract was centrifuged at 5000 g for 10 min and the supernatant was collected. This extraction was conducted twice and the supernatants were combined and deproteinated by the Sevag procedure. The supernatant was concentrated to volume of around 100 ml using a rotary evaporator (Buchi Labortechnic AG, Flawil, Switzerland) at 50 °C and 400 ml of 100% ethanol was added to the concentrate with stirring at 25 °C to form a suspension. The suspension was placed at 4 °C overnight. The crude polysaccharide was obtained by filtering the solution through a nylon membrane (0.45 μm pore size, Whatman International, Maidstone, UK), this being followed by washing with ethanol (99%) and acetone, and drying overnight at room temperature.

### 2.3. Chemical composition analysis

The total carbohydrate content of each sample was determined by the phenol-sulfuric acid method using glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Nitrogen content was determined by NA2100 Nitrogen and Protein Analyzer (Thermo Quest, Milan, Italy), and then protein content was obtained by a conversion factor of 5.41 (Oomah, Kenaschuk, Cui, & Mazza, 1995). The total uronic acid content was colorimetrically determined by the m-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973) using galacturonic acid as the standard.

Monosaccharide composition analysis was conducted using a modification of the method of Cui, Wood, Blackwell, and Nikiforuk (2000). The sample was hydrolyzed in 1 M $H_2SO_4$ at 100 °C for 2 h and diluted 50 times. The diluted sample were passed through a 0.45 um filter and infected to a high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex-5500, Dionex corporation, Canada). Separations were achieved with isocratic eluent (100 mM NaOH) on a CarboPac PA1 column (250 × 4 mm I.D., Dionex Corporation, Canada) and a guard column (3 × 25 mm, Dionex Corporation, Canada) at a flow rate of 1.0 ml/min. The column system was cleaned after each analysis with 300 mM NaOH for 30 min. A post-column delivery solvent system of 600 mM NaOH at a flow rate of 1.0 ml/min was added to the HPAEC-PAD system. The amount of total sugar in different fractions was taken as the sum of the monosaccharides in each fraction.

### 2.4. Rheological measurement

#### 2.4.1. Steady flow behavior

The effect of AP concentration, temperature, pH and ionic strength on the AP viscosity was studied using in an ARES rheometer (TA Instruments, New Castle, DE), equipped with a cone and plate geometry plate (50 mm diameter with a gap of 0.046 mm, angle 4°) or parallel plate geometry (50 mm diameter with a gap of 1 mm). During the experiments, the shearing geometry was covered with paraffin oil in order to prevent water loss. The power-law model was used to fit the experimental flow curves of AP at various concentrations, with the formula:

$$\eta_s = K \times \gamma^{n-1}$$

where $\eta_s$ is the apparent viscosity (Pa s), $K$ is the consistency index, and $n$ is the flow behavior index.

*2.4.1.1. Determination of flow properties at different concentration.* AP dispersions were prepared at concentrations of 0.1%, 0.5%, 1% and 2% by dispersing the required amount in deionized water at room temperature. The cone and plate were used and the range of shear rate was from 0.01 to 800 $s^{-1}$.

*2.4.1.2. Determination of flow properties at different temperature.* A 1% AP dispersion was prepared as Section 2.4.1.1 then loaded to base plate of rheometer and allowed to equilibrate at as set-point temperature for 1 h in water bath. Experiments were carried out at 5, 15, 25, 35, 45, 55, 65, 75 °C respectively. The parallel plate was used and the range of shear rate was from 0.01 to 100 $s^{-1}$.

*2.4.1.3. Determination of flow properties at different salt concentration.* A 1% AP dispersion was prepared as Section 2.4.1.1. Monovalent (NaCl) and divalent ($CaCl_2$) were added to the dispersion to give final concentrations of 0.001–1 M. The viscosity measurements were performed at 25 °C as described above. The cone and plate were used and the range of shear rate was from 0.01 to 800 $s^{-1}$.

*2.4.1.4. Determination of flow properties at different pH.* A 1% AP dispersion was prepared as Section 2.4.1.1 and adjusted the pH values to around 2, 4, 6, 8, 10 by using 4 M NaOH and HCl before the viscosity measurement, the temperature was also controlled at 25 °C in a water bath.