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Research Article

Discussion and Analysis of *Flammulina velutipes Polysaccharides* Compositions, Molecular Weight and Monosaccharide Composition

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Abstract: The aim of the present work was to explore the composition, molecular weight and monosaccharide composition of *Flammulina velutipes polysaccharides* by DEAE Cellulose-52, High Performance Gel Filtration Chromatography (HPGFC) and HPLC with PMP derivative with the FVP isolated and purified from *Flammulina velutipes*. The results showed that *Flammulina velutipes polysaccharides* were composed of three kinds of polysaccharides; the molecular weight of FVP was composed of 4191338, 372779, 19002; the molecular weight of 372779, 19002, one of the biggest average molecular weight polysaccharides is neutral polysaccharide, the rest of the two kinds of polysaccharide composition of less average molecular weight is acidic polysaccharides and the proportion of two kind of acidic polysaccharides significantly greater than neutral polysaccharide, 44.31 and 37.76% respectively, only 17.93% of neutral polysaccharide. FVP was composed of Glc, Man, Gal, Xyl, Fuc; the highest content of Glc, followed by Gal and Man, Xyl and Fuc content is relatively low. The molar ratio was relatively 13.05:2.75:3.16:1.48:1.00. In conclusion, FVP contained the more Glc, the less Fuc and a small amount of galacturonic acid and glucuronic acid, which showed FVP may contain acidic polysaccharides.

Keywords: *Flammulina velutipes polysaccharides*, monosaccharide composition, molecular weigh

INTRODUCTION

Polysaccharide is one of the important forms of naturally occurring sugar. It usually consists of more than 10 monosaccharide bases which are connected by glycosidic bond (Abdel-Akher *et al*., 1952; Agrawal, 1992; Pang *et al*., 2007), including polysaccharides (Ikekawa *et al*., 1982), storage structure and biological activity of polysaccharide (Mallavadhani *et al*., 2006; Pang *et al*., 2007). Because bioactive polysaccharide has much important biological activity, it has been applied in many areas such as functional food, biological medicine, biological materials (Wasser, 2002; Zhang *et al*., 2007). Bioactive polysaccharide is usually composed of several hundred to several thousand monosaccharides polymerization, whose nature is completely different from monosaccharides and biological activity of polysaccharides are related to its monosaccharide composition, molecular weight and structure (Yan *et al*., 2004). *Flammulina velutipes Polysaccharides* (FVP) is the most abundant biological active compounds content of *Flammulina velutipes*, has the good immunity, antitumor, protecting liver and enhance memory (Leung *et al*., 1997; Zheng *et al*., 2005; Peng *et al*., 2005).

The objective of this study was to improve the efficient use of FVP and the *Flammulina velutipes* postpartum added value and supply theoretical basis by adopting reasonable technical means to analyze the monosaccharide composition, molecular weight and component.

EXPERIMENTAL METHOD

Materials and reagents: *Flammulina velutipes* were from Jinan Jinbao *Flammulina velutipes* planting cooperative.

Reagents: Absolute ethyl alcohol, normal butanol, acetone, active carbon, chloroform, papayotin, DEAE Cellulose-52 (pharmacia), glucan (sigma), 1-phenyl-3-methyl-5-pyrazolone (PMP), xylose, glucosamine, galactosamine (Acros O rganics), ACS/HPLC (Tedia), trifluoroacetic acid, glucose, mannose, galactose, rhamnose, fucose, glucuronic acid, galacturonic acid, ribose, arabinose.

Isolation and purification of polysaccharide fractions: In the present work, the extraction and isolation of FVP was performanced by boiling-water decoction and ethanol precipitation to yield crude polysaccharide (Chauveau *et al*., 1996; Yalin *et al*., 2006; York *et al*., 1986). The dried *Flammulina velutipes* were crushed into fine particles, extracted with 25 times

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of double-distilled water and centrifuged. The combined aqueous extracts were concentrated in a beaker for leaching by centrifugation at 4,500r/min for 20min. The supernate was concentrated in a rotary evaporator under reduced pressure. The concentrated solution was mixed with 80% alcohol followed by centrifugation at 4,500r/min for 20min. Then the Sevag method was used to remove protein components after re-dissolution of the crude polysaccharides (Bhandari *et al*., 1990; Miyazaki and Nishijima, 1982). The solution was then dialyzed against distilled water for 2 days and precipitated by adding ethanol until the concentration of ethanol reached 80%. The precipitate was collected by centrifugation and washed successively with absolute ethanol and acetone to give a light yellow powder.

DEAE cellulose-52 column chromatography analysis: For additional purification the FVP were subjected to DEAE-52 cellulose column chromatography, eluting with distilled water (Angyal *et al*., 1974). Each 50 mL eluted fraction was collected and the content of sugar was monitored using the phenol-sulfuric acid method (Liu *et al*., 2007; Zhang *et al*., 2001). Fractions 8~14 were combined, concentrated to 100 mL and designated as the APS fraction. Then, this material was subjected to Sephadex G-150 column chromatography (2×60cm), eluting with distilled water collecting 50 mL fractions with monitoring by the phenol-sulfuric acid method.

HPGFC analysis: Chromatographic condition.

Chromatographic column: Ultrahydrogel™ Linear 300 mm×7.8mmid×2; Mobile phase: 0.1N sodium nitrate; Flow rate: 0.9ml/min; Column temperature: 45°C

Sample preparation: Sample (100mg) was dissolved in the mobile phase and set the volume to 25mL.

Drawing Standard Curve: Were injected Dextran T-5, Dextran T-10, DextranT-50, DextranT-150 standard solution.

PMP-HPLC analysis: PMP-HPLC was carried out on an Agilent-1100 HPLC system equipped with a quaternary gradient unit and a refractive index detector (RID). The analytical column used was a ZORBAX Eclipse XDB-C_{18} (250×4.6 mm i.d., 5μm) with 0.1 mol/L phosphate as the eluant at column temperature 30°C. The wavelength for RID detection was 250nm. Elution was carried out at a flow rate of1mL/min at 30°C.

The preparation of PMP-polysaccharide derivations was as follows (Fu and O'Neill, 1995; Fu and O'Neill, 1995; Li *et al*., 2003): Freeze-dried polysaccharides (2mg) were dissolved in 1mL 2 M TFA (trifluoroacetic acid). N_2 was bubbled through the solution for 30 s and then the ampoule was sealed. After hydrolysis for 6-8 h rat 110°C in an oven, the solution was cooled to room temperature. The collected peak elutes were dried via rotary evaporation to remove residual TFA at 50°C and then neutralized by 0.3 M NaOH to make 5 mL water sludge mixture. After that, 300 uL 0.5 mol/L PMP and NaOH were added and heated at 70 for 30 min. The solution was cooled to room temperature and neutralized with addition of 300 uL of 0.3 mol/L HCl. One milliliter chloroform was added to the mixture, shaken thoroughly, centrifuged and the super-natant was collected. Finally, the supernatant was filtered through 0.45-um filters to eliminate dust particles.

RESULTS AND DISCUSSION

Elution curve of FVP: As can be seen from the Fig. 1, elution with distilled water obtained a neutral polysaccharide; elution with 0.1~0.5 mol/L NaCl obtained an acidic polysaccharide and appeared two peaks.

Figure 2 shows the chromatograms peak of retention time (tR) of the four kinds of different molecular weight dextran. Based on the above data obtained regression equation: the Dextran calibration curve of was:

$$Lg (Mw) = - 0.2253tR+11.279, R^2 = 0.9948$$

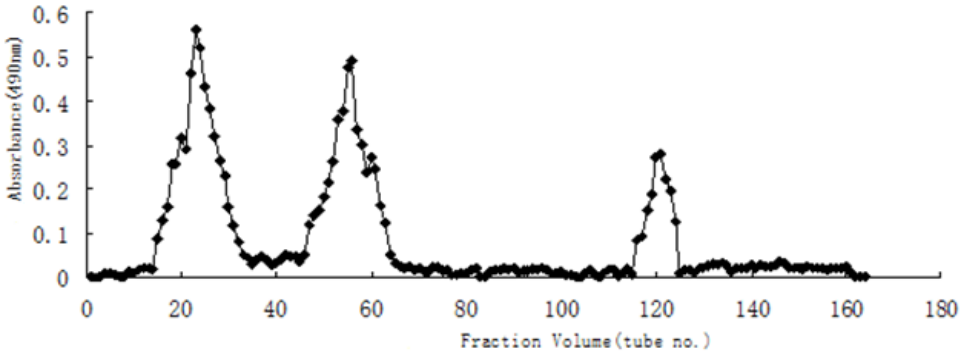

Fig. 1: Elution curve of FVP

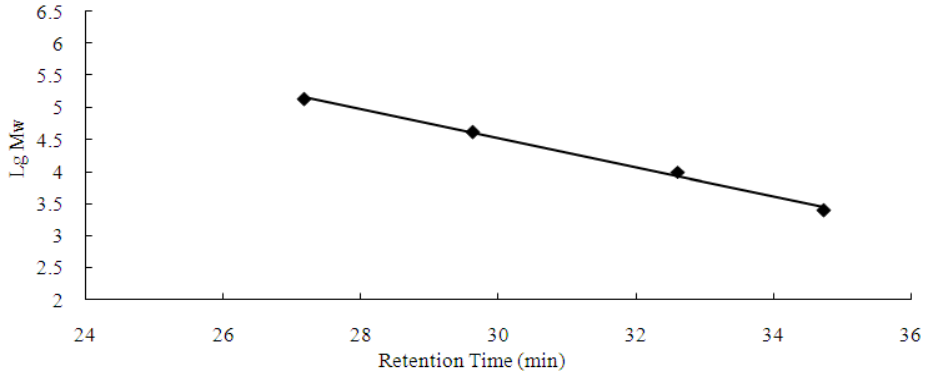

Fig. 2: The standard curve represented by dextran

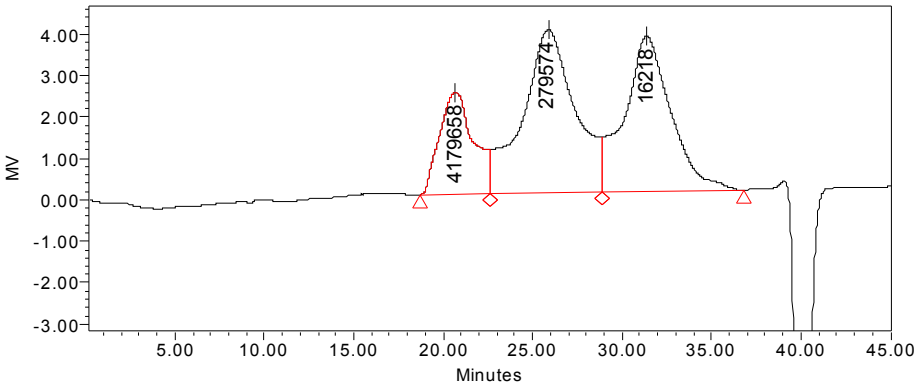

Fig. 3: The chromatogram of the FVP by HPGFC

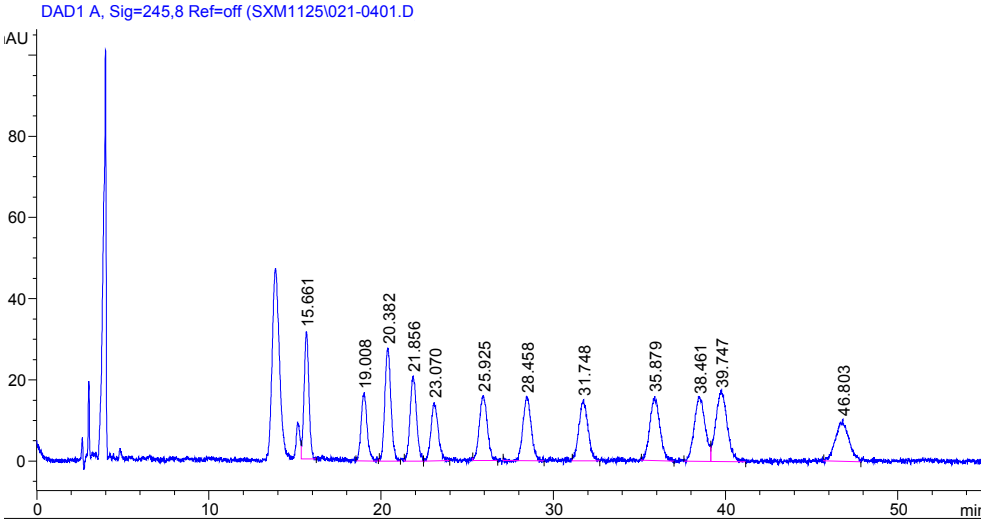

Fig. 4: HPLC of the mixed standard monosacharides

Table 1: Analytical results of the FVP by HPGFC

Peak No.	t_R(min)	Mn	Mw	MP	Area	The proportion of peak area (%)
1	20.671	3450347	4191338	4179658	325508	17.93
2	25.883	212168	372779	279574	804318	44.31
3	31.371	11281	19002	16218	685354	37.76

A HPGFC method was used to determine the molecular weight (Casu, 1982). Figure 3 shows the chromatograms of three kinds of polysaccharide compositions. Table 1 shows that the retention time

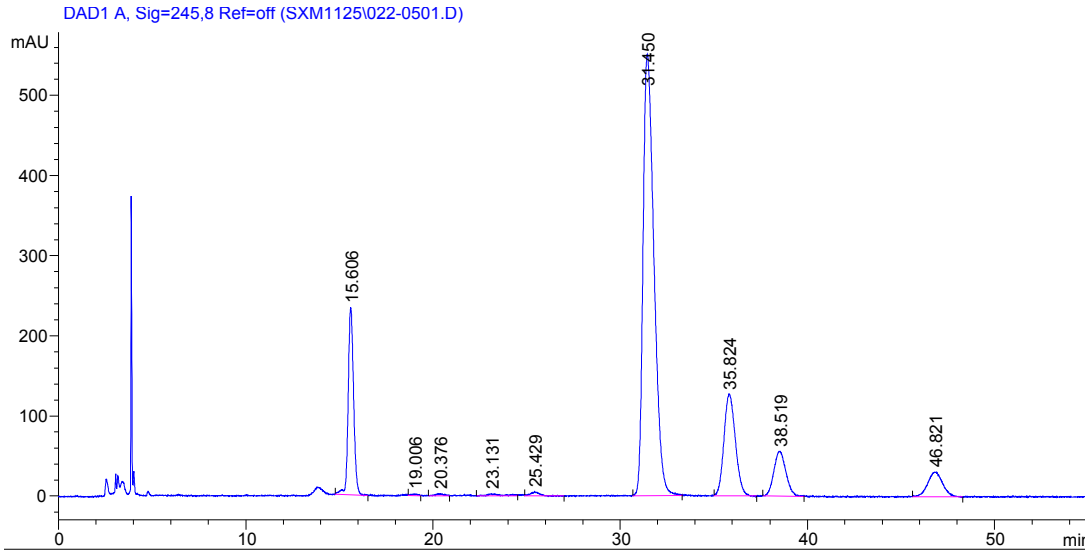

Fig. 5: HPLC of monosacharides of FVP

Table 2: The peak time of standard monosacharides

Monosacharides	The peak time (min)
Man	15.661
GlcN	19.008
Rib	20.382
Rham	21.856
GlcUA	23.070
GaiUA	25.925
GaiN	28.458
Glc	31.748
Gal	35.879
Xyl	38.461
Ara	39.747
Fuc	46.803

Table 3: Monosacharides of FVP and molar ratio

Monosacharides	t_R (min)	Peak area %	Molar ratio
Man	15.606	12.6717	2.75
Glc	31.450	60.1374	13.05
Gal	35.824	14.5636	3.16
Xyl	38.519	6.8099	1.48
Fuc	46.821	4.6069	1.00

(tR) of three polysaccharide components were 20.671 min, 25.883 min and 31.371 min, molecular weights were 4191338, 372779, 19002, peak areas were 325508, 804318, 685354. Among them the two of the smaller component proportions of the peak area were 44.31 and 37.76% and were larger than others.

The differences of column chromatography elution process, pretreatment of polysaccharide and protein removal process can lead to the differences of polysaccharide components. Of course, the differences of *Flammulina velutipes* species and origins may also lead to the differences of polysaccharide components.

The liquid chromatogram of standard monosaccharide: Figure 4 shows that different standard samples of monosaccharides in the chromatographic conditions can be clearly separated. Samples of the standard monosaccharides peak time are shown in Table 2.

HPLC of monosacharides of FVP: The chromatogram in Fig. 5 shows that using an HPLC method, FVP was composed of five monosaccharides-Man, Glc, Gal, Xyl and Fuc–in a molar ratio of 13.05:2.75:3.16:1.48:1.00. Table 3 shows that the retention time were 15.606, 31.450, 35.824, 38, 519, 46.821 and the corresponding peak area were 12.6717, 60.1374, 14.5636, 6.8099, 4.6069. The FVP composition of polysaccharides in Glc content is higher, the second is the Gal and Man and Fuc content is low. Thus infer, FVP mainly be consisted by dextran and galactose glycan, mannan, xylose chitosan, fucus chitosan and other components may be mixed in it.

CONCLUSION

According to DEAE Cellulose -52 column chromatography analysis, it shows that the FVP was composed of three polysaccharide components. HPGFC Analysis shows that the molecular weights were 4191338, 372779, 19002,. With heavy molecular weight as 4191338 polysaccharide was neutral polysaccharide, the rest of the two polysaccharides were acidic polysaccharides. Among them, two of the acidic polysaccharides were accounted for a higher proportion, 44.31 and 37.76% and only 17.93% neutral polysaccharide components.

FVP was composed of five monosaccharides-Man, Glc, Gal, Xyl and Fuc-in a molar ratio of 13.05:2.75:3.16:1.48:1.00. Can be concluded that FVP mainly be consisted by dextran and galactose glycan, mannan, xylose chitosan, fucus chitosan and other components may be mixed in it.

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