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Analytical Methods

# Characterization of a heterogalactan: Some nutritional values of the edible mushroom *Flammulina velutipes*

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**Abstract**

Production and consume of mushrooms have grown in the world, and beside these, the nutritional properties and biological active components of fungi have received more attention by researchers. Considering these, a mannofucogalactan was isolated from *Flammulina velutipes*, and characterized using $^{13}C$ and $^{1}H$ (obs.), $^{13}C$ HMQC nuclear magnetic resonance spectroscopy and methylation analysis. The monosaccharide composition of this polymer was determined by GC–MS and showed Fuc*p*, Man*p*, and Gal*p* in the molar ratio 20:16:64, respectively. $^{13}C$ NMR and $^{1}H$ (obs.), $^{13}C$ HMQC indicated an anomeric region containing signals (C-1/H-1) at $\delta$ 102.9/5.19, 102.0/5.16, and 98.8/5.05 corresponding, sequentially, to non-reducing end of α-D-Man*p*, 3-*O*-substituted α-L-Fuc*p*, and 6-*O*- and 2,6-di-*O*-substituted α-D-Gal*p* units. Along with methylation analysis, these data showed a structure with a main chain composed of 6-*O*-substituted Gal*p* units, partially substituted at O-2 by 3-*O*-D-mannopyranosyl-L-fucopyranosyl, α-D-mannopyranosyl, and in a minor proportion, α-L-fucopyranosyl groups. Furthermore, some nutritional values of this edible mushroom were evaluated, like amino acid and mineral nutrient contents.


*Keywords:* *Flammulina velutipes*; Mushroom; Heterogalactan

## 1. Introduction

Fungi have been utilized, for many years, in Asian countries like a very nutritive food and medicine. In 1978, the world mushroom production was about 1.1 million tones, and this production rose to 6.16 million tones, in 1997 (Chang, 2005). Now, consume of mushrooms are growing worldwide due to influence of oriental culture and studies about their nutritional values and pharmacological properties (Guterrez, Mantovani, Eira, Ribeiro, & Jordão, 2004; Yang, Lin, & Mau, 2002). Researchers are considering the edible basidiomycetes like health foods because they are good sources of vitamins, minerals, proteins, carbohydrates, high amounts of fibers and they are poor in fat (Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999). Considerable amounts of the essential amino acids and minerals like potassium, calcium and magnesium were observed in various species of *Pleurotus* (Akindahunsi & Oyetayo, 2006; Manzi et al., 1999). From many studies about these basidiomycetes, a number of fungal components have demonstrated biological activities, and among these, polysaccharides have received more attention as therapeutic molecules. An example are β-glucans, commonly extracted from mushrooms, which could present, normally, backbones of β-(1 → 3)- and/or β-(1 → 6)-linked D-glucopyranosyl units. These molecules showed biological effects, like protection against free-radical oxidation (Toklu et al., 2006), increased host responses against tumor or

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infections (Brown & Gordon, 2003; Leung, Fung, & Choy, 1997; Zheng, Jie, Hanchuan, & Moucheng, 2005) among others.

There are few studies about heteropolysaccharides extracted from macrofungi. Some of them, which are described as heterogalactans, heteromannans, and heteroglucans (Schepetkin & Quinn, 2006), have been tested in vitro and/or in vivo, and demonstrated biological activities like proliferation of lymphocytes, antitumor, and/or anticoagulant activities (Cho, Koshino, Yu, & Yoo, 1998; Ikekawa et al., 1982; Yoon et al., 2003).

*Flammulina velutipes* (Curt. ex Fr.) Sing. is one of the most popular edible mushrooms in Japan, and its morphological features are influenced by the incidence of light and temperature (Sakamoto, Ando, Tamai, & Yajima, 2007; Sakamoto, Tamai, & Yajima, 2004). Glucans and some heteropolysaccharides were isolated from this basidiomycete and some of them presented antitumor activity (Ikekawa et al., 1982; Leung et al., 1997; Mukumoto & Yamaguchi, 1977; Yoshioka, Sano, & Ikekawa, 1973). Smiderle et al. (2006) characterized a xylomannan from *F. velutipes*, that consisted of a main chain of (1 → 3)-linked α-Man*p* units, partially substituted at *O*-4 with single unit side chains of β-Xyl*p*, or of β-Xyl*p*-(1 → 3)-β-Xyl*p* groups. We now have fractionated extracts of *F. velutipes* fruiting bodies, obtained and characterized structurally a heteropolysaccharide described as a mannofucogalactan. Beside these, some nutritional values of this mushroom were analyzed.

## 2. Experimental

### 2.1. General experimental procedures

All solutions were evaporated at <40 °C under reduced pressure. Centrifugation was carried out at 9000 rpm for 15 min, at 25 °C. Alditol acetate mixtures were analyzed by GC–MS using a Varian model 3300 gas chromatograph linked to a Finnigan Ion-Trap, model 810-R12 mass spectrometer, using a DB-225 capillary column (30 m × 0.25 mm i.d.) programmed from 50 to 220 °C at 40 °C/min, then hold. Partially *O*-methylated alditol acetate mixtures were similarly analyzed, but with a program from 50 to 215 °C at 40 °C/min. The homogeneity and molar mass of 30E fraction were determined by high-performance size-exclusion chromatography (HPSEC–MALLS), using a Waters 510 HPLC pump at 0.6 ml/min, with four gel permeation columns in series with exclusion sizes of $10^6$–$5 \times 10^3$ Da, using a refractive index (RI) detector. Poly (ethylene oxide) of $M_w = 11{,}600$ was used as standard to calibrate the columns. The eluent was 0.1 mol/l aq. $NaNO_2$ with 200 ppm aq. $NaN_3$. Eluted fraction (30E) was dissolved in the eluent, filtered in membrane (0.22 μm), and injected (100 μl loop) at a 1 mg/ml concentration.

### 2.2. Fungal material

*F. velutipes* is recognized by a sticky, orange-brown cap of 1–3 cm broad and dark, finely pubescent stipe of 1.5–7 cm tall and 0.2–0.7 cm thick. The basidiomycete was purchased at the Public, Municipal Market of Curitiba, State of Paraná (PR), Brazil, and was identified by the Prof. Dr. Fábio Rosado from the Centro Universitário de Maringá (CESUMAR), Maringá-PR.

### 2.3. Polysaccharide extraction and purification

The dried fungus (88 g) was milled and submitted to aqueous extraction (3× at 100 °C, 1000 ml).

The aqueous extract was evaporated to a small volume and polysaccharide precipitated by addition to excess EtOH (3:1, v/v). The product was dialyzed against tap water for 48 h, concentrated under reduced pressure and freeze-dried. The extract was then dissolved in water and the solution submitted to freezing followed by mild thawing at 4 °C. The soluble fraction (SW), following centrifugation, was treated with Fehling solution (100 ml) and the insoluble $Cu^{2+}$ complex formed was isolated by centrifugation. Both complex (FP) and supernatant (FS) were neutralized with HOAc, dialyzed against tap water and deionized with Dowex 50 × 8 ($H^+$ form) ion-exchange resin (Fig. 1).

### 2.4. Monosaccharide composition

Each fraction (1 mg) was hydrolyzed with 2 M TFA at 100 °C for 8 h, followed by evaporation to dryness. The res-

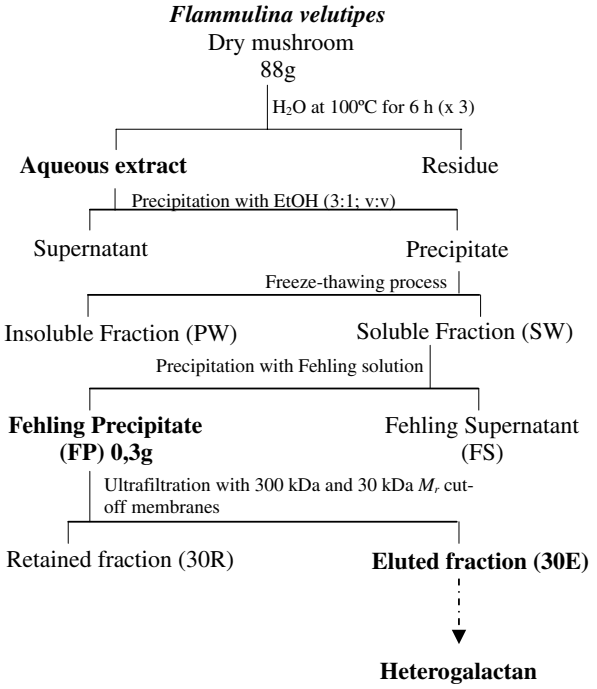


Fig. 1. Extraction and purification of heterogalactan (30E).