Mushrooms are consumed due to their taste, nutritional value and mainly because of the medicinal properties. The medicinal mushroom *Grifola frondosa* is among the most promising natural sources of substances that have been isolated and evaluated according its therapeutic effects. The polysaccharides are responsible for most biological effects attributed to mushrooms. In this way, carbohydrates from mycelium of this basidiomycete were analyzed. Alkaline extraction (KOH), purification by freeze-thawing and precipitation with Fehling solution were used to isolate heteromannan, which was structurally characterized by chemical derivatization methods such as methylation and Smith’s controlled degradation. Analytical techniques such as NMR, HPSEC-MALLS and GC-MS were also employed. A highly branched fucoxylomannan were proposed due to high levels of non-reducing terminal xylose (2,3,4-Me3Xyl, 16.5%), fucose (2,3,4-Me3Fuc, 13.9%) and mannose (2,3,4,6-Me4Man, 12.0%). The propose is in accordance with the high percentages of Manp3,4-di-O-substituted (2,6-Me2Man, 34.5%) of the main chain. The 3-O-(2,4-Me2Xyl, 2.0%), 4-O-(2,3-Me2Xyl, 13.1%) and 2,3-di-O-(4-MeXyl, 8.0%) Xylp units were also observed. The 13C NMR spectrum showed signals in anomeric region corresponding to β-Xylp (δ 103.6 and 101.2), α-Manp (δ 101.5, 101.0 and 100.7) and α-Fucp (δ 99.4 and 98.9) units. Substitutions at O-3 and O-4 of Xylp units were confirmed by the signals at δ 86.6 and δ 78.4, respectively. The signal at δ 78.2 refers to C-3 from α-Manp unit. The isolated heteropolysaccharide has a backbone of α-Manp-(1→3) linked and substituted by O-4 side chains constituted by Xylp, Fucp and Manp. According our knowledge, the isolated polymer was not described for basidiomycetes.

Keywords: *Grifola frondosa*, heteropolysaccharide, mycelium

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