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Review

D-Glucans from edible mushrooms: A review on the extraction, purification and chemical characterization approaches

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ABSTRACT

D-Glucans from edible mushrooms present diversified chemical structures. The most common type consists of a backbone of β-D-glucose (1 → 3)-linked frequently branched at O-6 by β-D-glucose residues as side chains. However it is possible to distinguish α-, β- and mixed D-glucans. Further discrimination could be made on the basis of glycosidic bond position in a pyranoid ring, distribution of specific glycosidic bonds along the chain, branching and molecular weight. The present manuscript reviews the processes of extraction, purification and chemical characterization of D-glucans, such as NMR studies, methylation analysis, Smith degradation, and some other methodologies employed in carbohydrate chemistry characterization. In addition, these polysaccharides are important because they can provide many therapeutic benefits related to their biological activity in animals and humans, either immunostimulatory activity, inhibiting tumor growth, as well as exerting antinociceptive and anti-inflammatory action, among others, which are usually attached to their structure, molecular weight and degree of branching.

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1. Introduction to mushroom D-glucans

Mushrooms have been appreciated and consumed for their nutritional value and medicinal properties in oriental countries for more than 2000 years. These fungi have traditionally been used for the prevention and also treatment of a multitude of disorders, and they have been increasingly consumed by cancer patients, during their treatments, as dietary supplements (Moradali, Mostafavi, Ghods, & Hedjaroude, 2007; Hardy, 2008). Researchers have considered these fungi as healthy food because they are good sources of vitamins, minerals, proteins, and carbohydrates, apart from low level of lipids and low caloric content (Wasser, 2002; Kalac, 2009). Fungi are excellent sources of D-glucans. They can present α-D-glucans, β-D-glucans, and also α/β-D-glucans in their fruit bodies (Synytsya & Novák, 2013). Mostly of these homopolysaccharides are not digested by human enzymes, being considered as fibers, which can assist intestinal motility and increase stool bulk, decreasing absorption of harmful toxic and carcinogenic substances, leading to lower incidence of cancer (Cheung, 2013).

Despite of the fact that mushroom D-glucans have presented to possess a variety of therapeutic benefits, the study on their chemical structures still requires more attention, especially in the fields of purification and chemical characterization. It was observed that the differences among the D-glucan structures may affect their biological properties (Giavasis, 2014). The glucans isolated from basidiomycetes present different linkage types, branching degrees, molecular weight, and solubility, therefore their chemical structures should be carefully determined with the aim of finding the best chemical structure for the desired therapeutics.

There are a plenty of methods to extract, purify and characterize these polysaccharides. This review attempts to summarize the methods for obtaining purified or crude D-glucans, as well as the techniques used for their chemical characterization and also to revise the structures of the D-glucans isolated from edible mushrooms up to this date. For this purpose, a search was performed using the Science Direct database, using the keywords combination: basidiomycete + homopolysaccharide + glucans, from 2004 to 2014. The most relevant findings were reported herein, as well as some classical articles about chemical reactions still used in carbohydrate analysis.

2. D-Glucans conventional extraction methods

The identification and characterization of carbohydrate structures requires, the isolation and purification of the polymer of interest from its original source. Regarding mushroom polysaccharides, they could be obtained from fruit bodies, mycelial biomass or directly from the liquid culture broth, as released exopolysaccharides (EPS). Besides that, spores and sclerotium from mushrooms also could be considered as sources for the extraction of glucans (Dong et al., 2012; Wang et al., 2014).

In general, D-glucans are commonly extracted using water in different temperatures or alkaline aqueous solutions. Therefore, these types of extraction will be covered in sequence.

2.1. Aqueous extraction

Powdered fruit bodies can be firstly extracted with some organic solvent, generally EtOH, acetone, or mixtures of $CHCl_3$:MeOH, to remove apolar compounds such as lipids, phenols and terpenes, although this is not demanded. The organic solvent extraction facilitates the complete separation of polysaccharides from other compounds that may be present (Carbonero et al., 2006; Dore et al., 2007; Smiderle et al., 2008a; Bi et al., 2009; Synytsya et al., 2009; Wang, Yu, & Mao, 2009; Klaus et al., 2011). The polysaccharide extraction is, traditionally, carried out with water at room temperature (Santos-Neves et al., 2008a,b; Smiderle et al., 2008a; Palacios, Garcia-Lafuente, Guillamón, & Villares, 2012; Smiderle et al., 2013). Depending on the aim of the study, this procedure could be repeated to exhaustion, in order to do a previous step of polysaccharide fractionation. After centrifugation, the supernatant, named cold water extract is separated from the residue. Therefore, the residue can be extracted with boiling water and again separated from the hot water extract by centrifugation (Santos-Neves et al., 2008a,b; Smiderle et al., 2008a; Palacios et al., 2012; Smiderle et al., 2013).

The exhaustive repetition of the extraction procedure contributes to enhance the yield of polysaccharide fractions, besides it diminishes the mixture of polysaccharides regarding the different extracts.

More advanced technologies to obtain polysaccharides have been used recently, as ultrasonic (Chen, Wang, Zhang, & Huang, 2012) and microwave assisted extractions (Zeng, Zhang, Gao, Jia, & Chen, 2012), besides the pressurized solvent extraction (Palanisamy et al., 2014). The latter procedure showed to be faster and more efficient in obtaining higher yield of polysaccharides, comparing to the traditional methodologies. Although it requires more specific devices, which increases the costs (Palanisamy et al., 2014).

2.2. Alkaline extraction

The remainder residue from aqueous extraction may be subsequently extracted with aqueous basic solutions (NaOH or KOH, 2% w/v) at 100 °C, but these conditions could vary (Smiderle et al., 2006; Amaral et al., 2008; Santos-Neves et al., 2008b; Synytsya et al., 2009; Chen, Zhang, & Cheung, 2010; Palacios et al., 2012; Song & Du, 2012; Ruthes et al., 2013a; Maity et al., 2014). Again, the residue is separated by centrifugation and the supernatant give rise to the alkaline extract. Extraction with basic aqueous solutions frequently is done using $NaBH_4$ to protect reducing end-units avoiding degradation of polysaccharide chains (Smiderle et al., 2006; Santos-Neves et al., 2008b).

3. Purification methods

After polysaccharide extraction, samples could be submitted to several purification steps to remove other substances such as proteins, phenolic compounds, monossacharides, amino acids or other related molecules. Proteins can be removed by precipitation with trichloroacetic acid (20%, w/v), by treatment with the enzyme protease at 40 °C for 1 h (pH 7.5), using Sevag method (Staub, 1965), or by treatment with phenolic reagent (Synytsya et al., 2009).

Moreover, several purification steps must be carried out in order to obtain pure polysaccharide fractions. The most common purification process used to obtain pure D-glucan fractions are listed below.

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