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# Deproteinization of water-soluble ß-glucan during acid extraction from fruiting bodies of *Pleurotus ostreatus* mushrooms

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ABSTRACT

Some ß-glucans can be easily extracted from *Basidiomycete* mushrooms but commonly used extraction procedures are not satisfactory. A simultaneous method for acid extraction and deproteinization in the case of *Pleurotus ostreatus* was developed using response surface methodology. The optimized extraction conditions proposed here (30 °C, 3.8% HCl, 300 min, stirring) allow for the simultaneous extraction and deproteinization of polysaccharides. Additionally, the acid extraction yield was 7 times greater than that of hot water extraction. The combined enzymatic digestion with lyticase, ß-glucanase, *exo*-1,3-ß-D-glucanase, and ß-glucosidase results elucidated that an extract containing ß-1,3-ß-1,6-ß-1,4-glucan. The gel permeation chromatography (GPC) results showed that the two glucan fractions obtained do not contain linked proteins. The weight average molecular weight of the first fraction ($M_w$ = 1137 kDa) was 60 times higher than that of the second fraction ($M_w$ = 19 kDa).



## 1. Introduction

The *Basidiomycete* mushroom *Pleurotus ostreatus*, commonly called the oyster mushroom, is the second most extensively cultivated species worldwide, after *Agaricus bisporus* (button mushroom) (Pérez-Martínez, Acevedo-Padilla, Bibbins-Martínez, Galván-Alonso, & Rosales-Mendoza, 2015). It is interesting as a source of biologically active glucans, which are the most numerous group of compounds among the mushroom's polysaccharides (Manzi, Aguzzi, & Pizzoferrato, 2001; Vetter, 2007). They exhibit antitumor effects, metastatic action, and antiatherogenic potential (Facchini et al., 2014; Ross, Větvička, Yan, Xia, & Větvičková, 1999; Tong et al., 2009; Vannucci, 2013). Research has also increasingly suggested that they may possess prebiotic properties (Synytsya et al., 2009). Moreover, glucans from mushrooms show excellent physicochemical properties, such as solubility, viscosity, and gelation, which are promising for commercial applications (Li, Zhang, & Xu, 2009). For these reasons, there is a strong tendency to commercially use mushroom biomass for the isolation of cellular glucans.

Structural studies have revealed that the glucans of *P. ostreatus* are mainly composed of branched β-(1,3), (1,6)-glucans (pleuran) and linear α-(1,3)-glucans (Synytsya et al., 2009; Tong et al., 2009). They can be linked to proteins, lipids, and other polysaccharides (Sarangi, Ghosh, Bhutia, Mallick, & Maiti, 2006). Their molecular weight, chemical composition, number of side chains branches, and spatial configuration all determine the physical and therapeutic properties of mushroom β-glucans (Chen & Seviour, 2007), such that even a slight difference in their basic structure provides them with individual properties (Chakraborty, Mondal, Pramanik, Rout, & Islam, 2004; Wasser, 2002). The exact characteristics of the chemical structure of fungal glucans therefore determine the possibilities of their therapeutic and biotechnological use. For instance, the strongest antitumor action is found for β-1,3-glucans having 1,6-linkages as branches with a triple helical conformation and molecular weight exceeding 1500 kDa (Ross et al., 1999; Wang et al., 2011).

There are the discrepancies in the scientific literature regarding the β-glucan content of mushrooms in general and of the oyster mushroom in particular. It has been reported that the concentration of β-glucans depends on the strain of *P. ostreatus* but falls within the range of 0.29–0.38 g/100 g dm (Manzi & Pizzoferrato, 2000); these values are in contrast to other studies, where the range of 3–9 g/100 g dm was indicated (Nitschke et al., 2011). The difference is related to the selection of effective methods for the isolation and purification of the β-glucan from the fungal biomass.

A simple procedure for the isolation, fractionation, and purification of polysaccharides from Macromycetes was developed by Mizuno et al. (1999). With some modifications, it is still used

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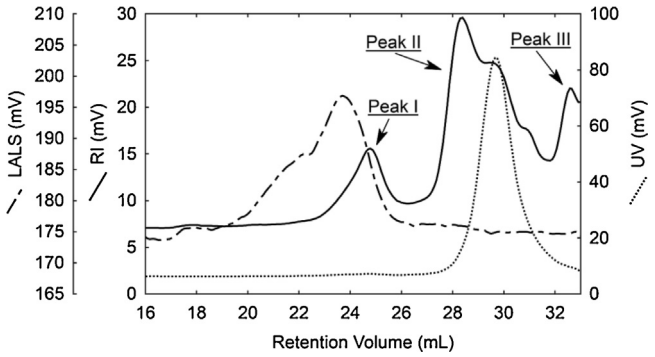


**Fig. 1.** GPC profile of ß-glucans extracted with water from *Pleurotus ostreatus* at 100 °C for 6 h.

nowadays. Briefly, it includes the elimination of low molecular weight substances (monosaccharides, disaccharides, polyphenols, vitamins, etc.) from the mushroom material using 80% ethanol. The alcoholic solvent also removes fats (Han et al., 2011). In next step, extractions with water (100 °C, 3 h), ammonium oxalate, and 5% sodium hydroxide are carried out sequentially (Mizuno et al., 1999). However, the effectiveness of these solvents is not satisfactory in solubilizing polysaccharides from mushrooms (Manzi & Pizzoferrato, 2000). Additionally, in further research on the characterization of mushroom glucans, both physicochemical and biological, the presence of protein in the polysaccharide fraction is also a serious problem, and one that can lead to false conclusions. Therefore, the proper characterization of these compounds requires deproteinization. The methods for removing free and linked proteins are, however, not effective enough. The free proteins are usually removed by the Sevage method or by dialysis (Synytsya et al., 2009; Yang & Zhang, 2009; Zha et al., 2012; Zhang, Li, Wang, Zhang, & Cheung, 2011). The Sevage methods offers only low-efficiency deproteinization at the cost of high losses of polysaccharides (Zha et al., 2012). Another way to remove the protein from the glucans is the use of proteolytic enzymes (Manzi et al., 2001). In this case, the deproteinization efficiency is much higher and there is a smaller, though still unsatisfactory, loss of polysaccharides (Zha et al., 2012). High-efficiency deproteinization is observed during hydrolysis involving trifluoroacetic acid. However, in this case, beside the desired protein losses, large losses of polysaccharides of up to 78% are observed (Zha et al., 2012). An effective means of polysaccharide deproteinization could also be applied to solid phase extraction techniques (Packer, Lawson, Jardine, & Redmond, 1998).

In this article, we give a new simultaneous acid procedure for isolation/extraction and deproteinization of water-soluble glucans (WSG) from the oyster mushroom *P. ostreatus* P80. The yield of this acid extraction is seven times higher than that of hot water extraction. Additionally, it provides total deproteinization without loss of the polysaccharide fraction. WSGs are particularly interesting for industry, because water solubility is a factor that significantly enhances both the biological activity of glucans (Wasser, 2002) and the clinical quality of preparations associated with the ability to permeate stomach walls after oral ingestion.

## 2. Materials and methods

### 2.1. Materials and reagents

The material consisted of milled dried *P. ostreatus* (Jacq.: Fr.) Kumm. obtained from fruiting bodies grown on wheat straw substrate spawned from a mycelium (cv. K-22). The culture was conducted at 13–15 °C and at a *relative humidity* of about 85–90%.

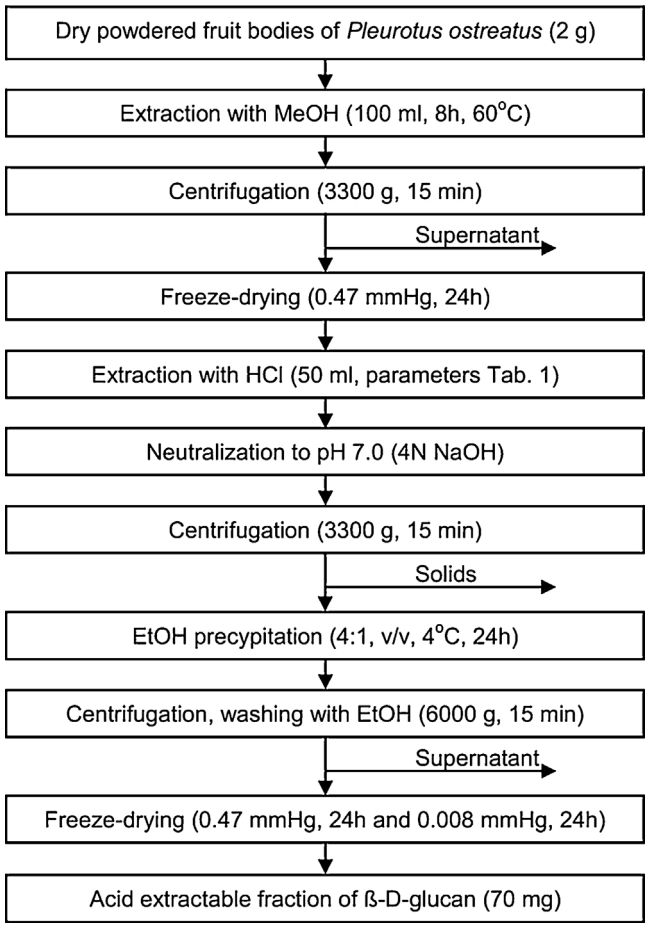


**Fig. 2.** Scheme for the extraction of ß-glucan obtained from fruiting bodies of *Pleurotus ostreatus*.

The fruiting bodies of the oyster mushroom *P. ostreatus* (Jacq.: Fr.) Kumm. were dried at 40 °C for 8 h at 70 °C. The dried mushrooms were milled with particle size smaller than 500 μm. Pullulan standards were from Shodex (Japan). Exo-1,3-ß-D-glucanase from *Trichoderma* sp. (EC 3.2.1.58), ß-glucosidase from *Aspergillus niger* (EC 3.2.1.21) and a yeast and mushroom β-glucan assay kit were purchased from Megazyme (Ireland). Lyticase from *Arthrobacter luteus* (CAS number 37340-57-1), ß-glucanase from *Bacillus subtilis* (EC 3.2.1.4), carbohydrate standards for maltose, glucose, fructose, xylose, and D-Glucose-$^{13}C_6$, and other reagents (hydrochloric acid, sodium hydroxide, acetonitrile, and sodium nitrate, trifluoroacetic acid) were obtained from Sigma-Aldrich (USA).

### 2.2. Enzymatic ß-glucan determination

The colorimetric method for the enzymatic determination of ß-glucan was used (procedure for yeast and mushroom ß-glucan assay kit, Megazyme, Ireland). Acid solubilization (10 N HCl) and extensive hydrolysis (1.3 N HCl at 100 °C, 2 h) were performed. The traces of laminarisaccharides were hydrolyzed with *exo*-1,3-β-glucanase and β-glucosidase. The glucose released was determined in the reaction mixture containing glucose oxidase plus peroxidase, 4-aminoantipyrine, and *p*-hydroxybenzoic acid; spectrophotometric measurement of quinoneimine dye was carried out at 510 nm. The determined free glucose was converted to anhydroglucose, as occurs in ß-glucan (a factor of 162/180).