

DOI:10.29013/AJT-25-5.6-78-85



## POLYSACCHARIDES OF PLEUROTUS OSTREATUS MUSHROOM

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**Cite:** Tursunova N., Kamolov L., Nomozova M. (2025). Polysaccharides of *Pleurotus Ostreatus* Mushroom. *Austrian Journal of Technical and Natural Sciences* 2024, No 5 – 6. <https://doi.org/10.29013/AJT-25-5.6-78-85>

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### Abstract

In this study, polysaccharide fractions extracted with water and precipitated with ethanol from the mushroom *Pleurotus ostreatus* growing in Uzbekistan were studied. The functional groups in the fractions were identified by IR spectroscopy and confirmed that they were mainly  $\beta$  – (1  $\rightarrow$  3) / (1  $\rightarrow$  6)-glucans. The biological activity of the obtained substance was based on its structural properties. Polysaccharides are the most famous and most potent substances derived from mushrooms that have antitumor and immunomodulatory properties. *Pleurotus ostreatus* polysaccharides –  $\beta$  – glucans – are also known to have antitumor activity, as they are known from the literature to strengthen the immune system and activate natural defenses against tumors.

**Keywords:** *Pleurotus ostreatus*, polysaccharides,  $\beta$  – glucans, IR spectrum, biological activity

### Introduction

*Pleurotus ostreatus* is a species of mushroom widely distributed in Uzbekistan and of nutritional and pharmaceutical importance. This mushroom contains biologically active polysaccharides such as  $\beta$  – glucans, which have immunomodulatory, antioxidant and antitumor properties. Studying the structure of these polysaccharides and evaluating their biological activity is a pressing issue for modern scientific research. Mushrooms have been part of the human diet for thousands of years in many Asian countries, such as China, Korea and Japan. It is known that mushrooms are valuable food for health, as they are rich in fats and proteins, vitamins and rare minerals. Currently, the beneficial effects of mush-

rooms are increasingly recognized and they are attracting great attention in food and pharmaceutical applications (Zhang Y. et al., 2012). Natural antitumor polysaccharides isolated from mushrooms include acidic and neutral polysaccharides with various glycosidic linkages, and some are linked to protein or peptide residues, such as polysaccharide-protein or peptide complexes. In addition to the primary structure, the higher structure of polysaccharides, such as chain conformation, also plays an important role in their antitumor activity. Most polysaccharides are classified as non-specific bioactive substances because their exact mode of action is unknown and the chain conformation of their active components has not been determined (Zhang, M.,

Cui, S. W., Cheung, P. C. K., & Wang, Q. 2007). Mushroom polysaccharides play an important role in functional foods because they also exhibit biological modulatory properties such as antiviral and antibacterial activities. The production, purification, and characterization of intracellular and extracellular free and protein-bound polysaccharides from *Pleurotus ostreatus* and their growth inhibitory effects on human carcinoma cell lines have been studied (Silva S. et al., 2012). Carbohydrates are one of the main nutrients of mushrooms, accounting for approximately 40–70% of the dry weight, including low and high molecular weight carbohydrates, with low molecular weight carbohydrates consisting primarily of monosaccharides, disaccharides, and sugar alcohols (polyols), such as glucose, trehalose, mannitol, and arabinol. High molecular weight carbohydrates are primarily polysaccharides such as glucans and chitin. Some of the carbohydrates abundant in mushrooms can be obtained for food, medical, and cosmetic purposes. For example, sugar alcohols contain relatively few calories and can be used as sugar substitutes in desserts and other foods. The hygroscopicity of trehalose makes it an ideal humectant for use in cosmetics. Fungal chitin and its deacetylated derivative chitosan have antimicrobial bioactivity and can be used as a wound healing agent. Finally, polysaccharides such as  $\beta$ -glucan have immunomodulatory activity by stimulating macrophages and other white blood cells (Zhou S. et al., 2016). Mushroom carbohydrates are obtained from compost substrates such as wood, straw, and straw, which contain approximately 60–70% cellulose and hemicelluloses. During mushroom growth, cellulose and hemicellulose are hydrolyzed by enzymes from the mycelium, transported into mycelial cells, and then metabolized through various carbohydrate pathways. In addition to being degraded by glycolysis to produce energy for growth, metabolites can be synthesized into structural polysaccharides of the cell wall, such as glucan and chitin. Metabolites can also be synthesized into glycogen, arabinol, trehalose, or mannitol for use in developmental or later growth phases. The distribution of carbohydrates in mushrooms varies significantly in different regions during growth and fruiting. The distribution of carbohydrates in the compost and fruiting

bodies of *Pleurotus ostreatus* was analyzed. When the amounts of sugars, polyols, polysaccharides and chitin were determined at different growth stages and in different parts of the fungus, trehalose, mannitol and glucose were first accumulated in the compost, and then decreased during the differentiation and growth of the fruiting body. Polysaccharides were mainly accumulated in the fruiting body and its adjacent regions of the fungus, and chitin was mainly observed in the basal region. These findings provide insights into the function and utilization of carbohydrates during fungal growth (Zhou S. et al., 2016). Of all the biologically active components, polysaccharides in particular,  $\beta$ -glucan is the most studied group of functional compounds in mushrooms.  $\beta$ -glucan is a long-chain polymer of glucose units linked together by glycosidic bonds, found in the cell walls of oats, barley, yeast, and fungi.  $\beta$ -glucans from different sources have different bond types, branching patterns, and molecular weights. In oats and barley, it consists of a linear glucose polymer with  $\beta$ -(1–3),  $\beta$ -(1–4) linkages, and  $\beta$ -(1–6) linkages. The  $\beta$ -glucan content in fungi ranges from 0.21 to 0.53 g/100 g (dry weight basis). Most of the fungal  $\beta$ -glucan is found as an insoluble fraction (54–82%) and only a small portion (16–46%) is found as a soluble fraction. Mushroom  $\beta$ -glucans are known as biological response modifiers (BRM), which are used in both modern medicine and traditional chemotherapeutic drugs to treat cancer and various infectious diseases (Khan A.A. et al., 2017). In fact,  $\beta$ -glucans are the main pathogen-associated molecular pattern recognized in fungal infections and responsible for triggering the immune response. The immune system Modulation by  $\beta$ -glucans is complex, involving many factors that are not yet fully understood (Pérez-Bassart Z. et al., 2023). There are over 200 clinical trials documenting their use for a range of applications. Since 1980, licensed drugs containing  $\beta$ -glucans have also been available on the pharmaceutical market in Japan for the treatment of cancer.  $\beta$ -glucans have been recognized as pharmaceutical agents in several countries, including the United States, Canada, Finland, Sweden, China, and Korea. The diverse functional effects of these molecules include modifying lipid and glucose metabolism, lowering

cholesterol, regulating obesity and reducing the risk of cardiovascular disease and diabetes, modulating the gut microbiome, modifying lipid and glucose metabolism, and beneficial effects on gastrointestinal diseases, etc.  $\beta$ -glucans, especially those derived from non-cereal sources, are widely known for their immunomodulatory properties, their ability to stimulate the immune response and initiate inflammatory properties, and their ability to resist infections.  $\beta$ -glucans derived from fungi are the most potent immune modulators. Patients with nosocomial pneumonia and When  $\beta$ -glucans were administered to prevent sepsis, the treatment group had a lower incidence of pneumonia, as well as a lower mortality rate, compared to the control group. was low (Murphy E. J. et al., 2022).

$\beta$ -glucans are used in the food industry as gelling agents and thickeners in the production of low-fat foods with improved texture (milk, bread or yogurt). In particular, hydrogels are three-dimensional, hydrophilic, polymeric networks capable of retaining large amounts of water. Furthermore,  $\beta$ -glucans constitute a soluble fiber with strong prebiotic effects. Although  $\beta$ -glucans can be easily extracted with water, more aggressive treatments are required for the upstream  $\beta$ -glucans (i.e., highly alkaline solutions, pH 13–14). However, water-soluble fractions may be a more environmentally sustainable option for the production of new functional ingredients. Furthermore, discarded whole biomass and uncommercialized residues (stipes) that do not meet commercial standards such as size and appearance may be cheap and abundant sources of these compounds (Pérez-Bassart Z. et al., 2024).

Water-soluble  $\beta$ -glucans from *Pleurotus ostreatus*, one of the most widely cultivated mushrooms, have been shown to be an environmentally sustainable option for the production of natural emulsifiers. It is assumed that the structure and composition of  $\beta$ -glucan-rich extracts depend on the source (whole biomass of *P. ostreatus*) and the extraction conditions (more or less purified samples), and therefore these aspects also affect their emulsifying properties. The composition of  $\beta$ -glucan-rich extracts and the presence of proteins or other small compounds have been shown to play an important role in the emul-

sifying properties of the obtained extracts (Pérez-Bassart Z. et al., 2024). Monounsaturated and polyunsaturated fatty acids such as linoleic, oleic and linolenic acids are used in skin care. The stems of this mushroom contain large amounts of ergosterol, which serves as a valuable hypocholesterolemic supplement by inhibiting cholesterol absorption into the blood and stimulating cholesterol secretion. Ergosterol can also be converted to vitamin D upon UV irradiation, which increases calcium absorption and is essential for the skeletal system in humans (Ayser M. et al., 2023). Unlike yeast, oat, and barley  $\beta$ -glucans, which have been shown to play a role in various cancers, the role and mechanism of *P. ostreatus*-derived  $\beta$ -glucans have been studied in the treatment of cervical cancer.  $\beta$ -glucan particles from *P. ostreatus* have also been isolated as drug carriers for the prevention and treatment of cancer. Their biological activity against cervical cancer was investigated and it was concluded that the modified particles can induce ROS-mediated apoptosis in cancer cells (Seifeldin S. A. et al., 2024). Developing an efficient extraction technology is crucial to ensure high yield while preserving the bioactive properties of the  $\beta$ -glucan component (Frioui M. et al., 2024). The anti-obesity benefits of  $\beta$ -glucan have been studied for a long time. Results show that  $\beta$ -glucan can improve hypertriglyceridemia, hypercholesterolemia, hypertension, hyperglycemia and insulin resistance (Nastiti A. et al., 2024). The immune profile of patients with endocrine-dependent breast cancer (clinical stages I–II) in clinical and imaging remission after pleura ( $\beta$ -glucan from *Pleurotus ostreatus*) administration has shown that long-term pleura administration may have potential benefits on antitumor cellular immunity in breast cancer patients in remission (Spacek J. et al., 2022). The functionality of  $\beta$ -glucans is related to their physicochemical properties, such as thickening, stabilizing, emulsifying, foaming, and gelling properties. These properties have been extensively studied for  $\beta$ -glucans from various cereal crops, but are not well understood for  $\beta$ -glucans from fungi. It has been found that  $\beta$ -glucans from yeast can be used as thickeners, water-retaining or fat-binding agents, and emulsifying stabilizers in food products. Since yeast

and fungal glucans share a common structure, it can be assumed that they also have similar physical and chemical properties (Gallotti F., Turchiuli C., Lavelli V., 2022).

### Method

Sample preparation:

*Oyster mushroom* The mushroom sample was collected from local areas. The dried and ground raw material was prepared for extraction.

#### Polysaccharide extraction:

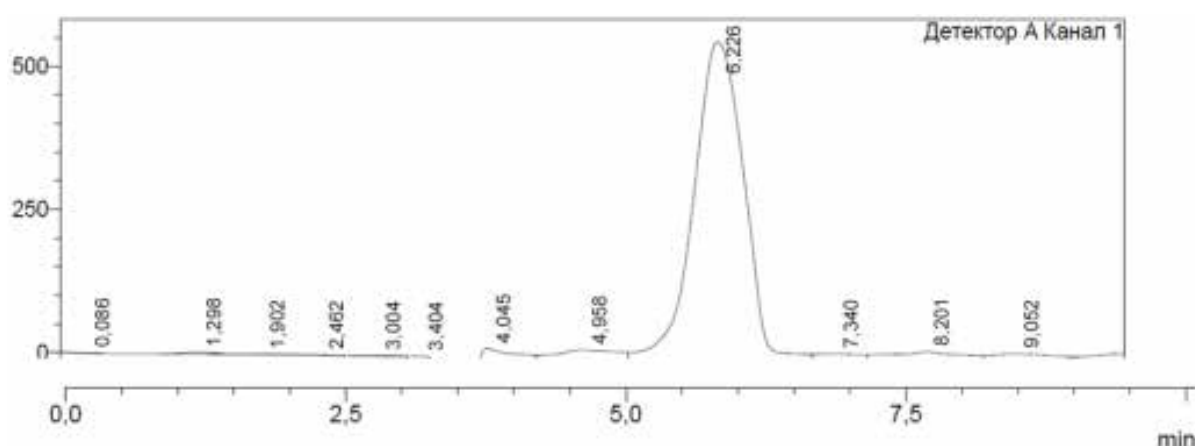
280 g of crushed *Pleurotus ostreatus* biomass was extracted with distilled water at room temperature of 25 °C for 24–48 h. The resulting extract was first filtered. The resulting extract was reduced in solvent using

vacuum. Then, it was mixed with 3 times the mass of cold ethanol, cooled for 12 hours and filtered. The residue remaining on the filter was squeezed using a cloth and dried at room temperature. As a result, 7 g of polysaccharide was obtained, which was 2.5% of the total biomass.

### Results and analysis

The carbohydrates obtained as a result of the extraction were subjected to high-performance liquid chromatography and the chromatographic analysis of the obtained fraction yielded the following results. High-performance liquid chromatography analyses of the extracted carbohydrates were performed (Figure 1).

**Figure 1.** Top layered liquid chromatography results



**Table 1.** High effective liquid chromatography the results

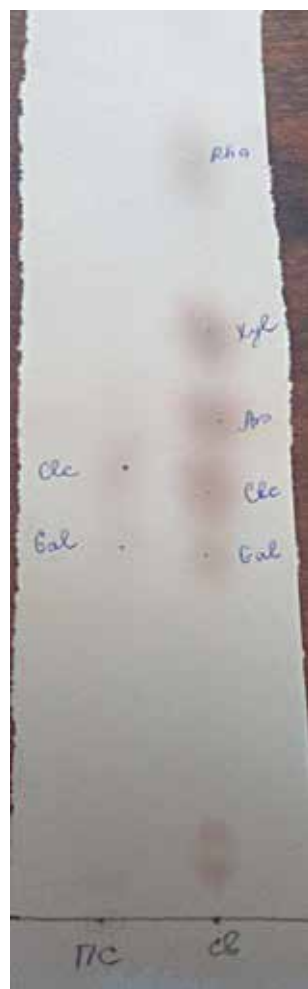
Детектор А Канал 1							
Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0,086	5605	370	0,000			
2	1,298	65233	3231	0,000			
3	1,902	15206	734	0,000			
4	2,462	6081	496	0,000			
5	3,004	17524	983	0,000			
6	3,404	1374396	35345	0,000			
7	4,045	5285764	160394	0,000			
8	4,958	6852857	134320	0,000		V	
9	6,226	26871600	638487	0,000		V	
10	7,340	1965940	65534	0,000		V	
11	8,201	2821988	46145	0,000		V	
12	9,052	794137	20917	0,000		V	
Сумма		46076331	1106957				

The results of the table above show that the signal emitted at a time interval of 6.226 minutes was seen to have the highest concentration of 638487 g/mol.

*Pleurotus ostreatus* mushroom biomass were performed by extraction with distilled

water at room temperature. In order to determine the structure of the isolated polysaccharide, acid hydrolysis and thin layer paper chromatography analysis were performed (Figure 2).

**Figure 2.** Polysaccharide paper chromatography



The following table was prepared by comparing the paper chromatography results with the standards (Table 2).

From the results shown in the table, it was confirmed that the polysaccharide was  $\beta$ -(1  $\rightarrow$  3)/(1  $\rightarrow$  6)-glucans.

To study their structure, IR spectra were obtained (Figure 3).

**Table 2.**

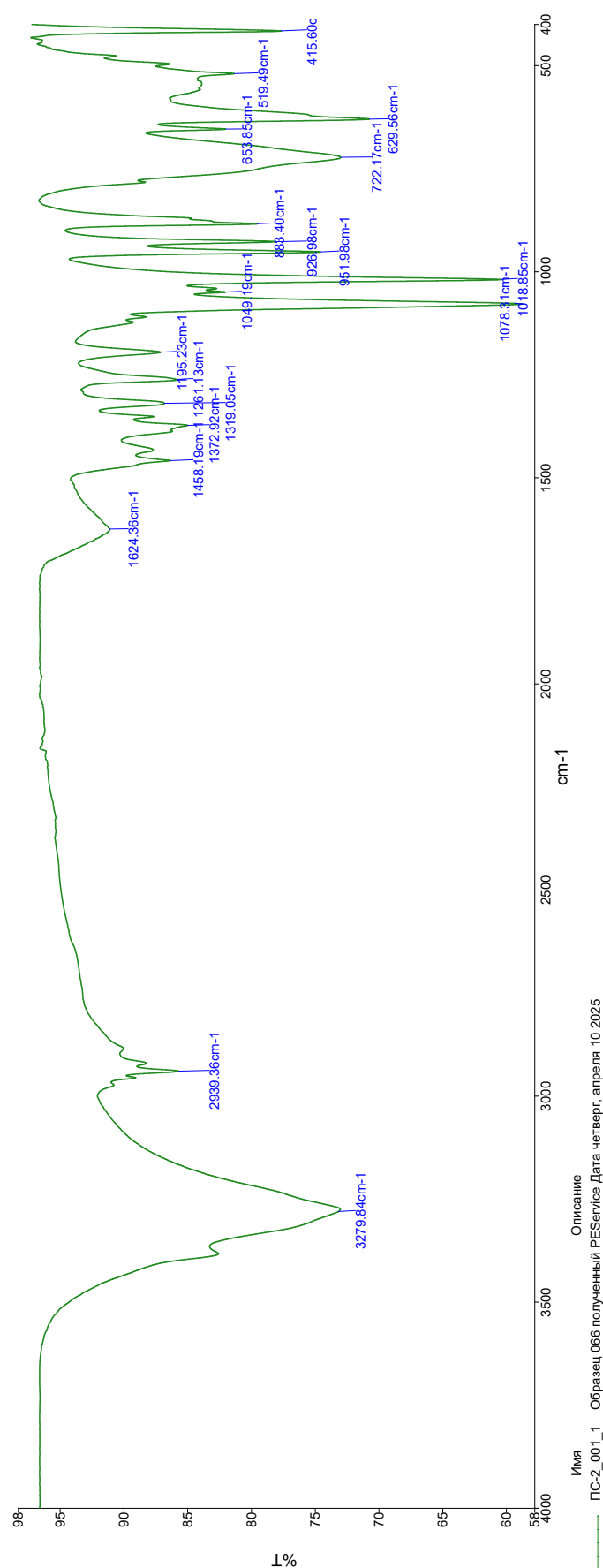
	Yield %	Quantitative monosaccharide composition					
		Galac- tose	glucose	Man- nose	Arabinose	Xelose	Ravnoza
Polysaccharide	2.5%	7	93	–	–	–	–

### 3. Pleurotus ostreatus mushroom polysaccharide IR spectrum

The following main lines were detected in the IR spectrum: a broad extended line characteristic of –OH groups around 3279.8  $\text{cm}^{-1}$ , 2939.36  $\text{cm}^{-1}$  – stretching vibrations characteristic of the  $\text{CH}_2$  – group are visible, 1624.36  $\text{cm}^{-1}$  – bound water molecules, and in the range of 1261–1020  $\text{cm}^{-1}$ ,

C–O–C and C–O–H stretching vibrations characteristic of  $\beta$  –glucans are visible. In the region of 961–650  $\text{cm}^{-1}$ , deformation signals characteristic of the CH- group are visible. These signals indicate that the polysaccharide is  $\beta$  –glucan.

**Figure 3.**





### Conclusion

This study is an important step in the study of the extraction of  $\beta$ -glucans from *Pleurotus ostreatus* mushrooms and their biological activity. The results obtained by IR spectroscopy and HPLC analysis allowed us to determine in detail the molecular structure of the polysaccharides, their purity and bioactive activity. These results provide the basis for considering *Pleurotus ostreatus*

mushrooms as a potential source for use in the pharmaceutical and food industries. The results obtained in the study indicate the need to study other bioactive properties of  $\beta$ -glucans, such as antioxidant and antibacterial activity. In the future, further study of these polysaccharides and the development of more efficient extraction methods will allow for their wider application in the pharmaceutical industry.

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submitted 17.04.2025;

accepted for publication 01.05.2025;

published 29.05.2025

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