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*Research article*

# EXTRACTION AND CHARACTERIZATION OF BETA-GLUCANS FROM DIFFERENT MUSHROOMS AND ALGAE

**Buse Ozdere Yilmaz[1], Neslihan Bozdogan[1], Bilge Basturk Berk[1], Seher Kumcuoglu[1], Sebnem Tavman[1]✉**

*[1]Department of Food Engineering, Faculty of Engineering, Ege University, Izmir, Türkiye*

*✉Corresponding author: Sebnem Tavman, ORCID: 0000-0002-3069-1709, +902323113016, E-mail: sebnem.tavman@ege.edu.tr*

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**Abstract**

β-glucans are bioactive polysaccharides with immunomodulatory and prebiotic functions. This study assessed the extraction efficiency and structural features of β-glucans from six edible mushroom samples and three algae. Quantitative analysis was done via enzymatic hydrolysis, and structural characterization employed FTIR and SEM. β-glucan content varied significantly; *Ganoderma lucidum, Lentinula edodes*, and *Pleurotus ostreatus* showed higher levels and distinct cell wall morphologies. Algal samples had lower content but unique structures. SEM revealed porous, fibrous walls enhanced extractability. Findings highlight mushrooms as rich β-glucan sources and suggest synergistic potential of algae in functional food formulations.

## 1. Introduction

Polysaccharides are biological macromolecules composed of repeating glucose units that exhibit structural differences from proteins, nucleic acids, glycoproteins, and glycolipids (Sandford & Baird, 1983). Variations in the length of polysaccharide chains result in a wide range of molecular weights (Cui et al. 2023). Excluding starch and its derivatives, most polysaccharides have gained importance in the food industry due to their secondary functionalities, such as emulsification, suspension, stabilization, encapsulation, flocculation, film formation, binding, and coating. Additionally, they positively influence food texture, flavor, color, and appearance (Sandford and Baird 1983).

B-glucans are prominent polysaccharides widely found in nature. These are glucose polymers consisting of D-glucose monomers linked via β-(1→2), β-(1→3), β-(1→4), and β-(1→6) glycosidic bonds, forming either branched or linear chains (Sarantis et al. 2021; Zhu et al. 2016). As biologically active polysaccharides, β-glucans are commonly present in yeasts, mushrooms, certain bacteria, algae, and cereal grains such as oats and barley (Du et al. 2014; Zhu et al. 2015; Du et al. 2019). Their chemical structures, molecular

weights, and functional properties vary depending on the biological source (Kaur and Riar 2020).

Cereal-derived β-glucans generally consist of linear β-(1→3) and β-(1→4) linkages, whereas those from fungi and yeasts feature a β-(1→3) backbone with β-(1→6) branching (Du et al. 2019). Cereal β-glucans exhibit similar molecular structures regardless of their source; these structures are formed by mixed arrangements of β-(1→3) and β-(1→4) bonds (Mejía et al. 2020; Schmidt 2022). Although cereal β-glucans share relatively uniform molecular structures regardless of the source, the ratio of glycosidic bonds, the length of cellulose fragments, and the proportion of cellotriose/cellotetraose units contribute to structural variations (Izydorczyk and Dexter 2008; Benito-Román et al. 2013a; Benito-Román et al. 2013b).

In mushrooms and algae, β-glucans serve as critical structural components. Edible and medicinal mushrooms typically contain complex β-glucans with a β-(1→3) backbone and β-(1→6) side chains that exhibit immunomodulatory and other bioactive effects (Du et al. 2014; Zhu et al. 2015; Du et al. 2019). For example, mushrooms such as *Ganoderma lucidum, Lentinula edodes*, and *Pleurotus ostreatus* are particularly rich in β-glucans. Algal β-glucans, in contrast, are generally more soluble and differ in glycosidic linkage patterns and functional group composition (Niu et al. 2015; Tsubaki et al. 2016; Mokhbatly et al. 2020). Notably, glucans from algae such as Ulva spp. and *Undaria pinnatifida* (wakame) are recognized for their prebiotic potential. B-glucans obtained from these two natural sources enable various functional food applications thanks to their structural differences.

The structural diversity of β-glucans not only depends on the source organism but also on factors such as monosaccharide composition, glycosidic linkage types, and chain conformations (Du et al. 2019; Boulaka et al. 2020). These differences significantly affect their functional and biological properties, including viscosity, solubility, gelation, and prebiotic activity. In particular, long-chain, high-molecular-weight β-glucans can form gel-like structures in aqueous environments and serve as natural thickeners in food formulations (Zhu et al. 2016).

Furthermore, β-glucans have demonstrated numerous health benefits, including lowering the risk of coronary heart disease (Sarantis et al. 2021), modulating immune responses, and promoting the production of short-chain fatty acids through fermentation (Cummings 1991; Cummings 1995; Hamer et al. 2008). In a study conducted by Cantu-Jungles et al. (2018), the fermentation profiles of two different β-glucan structures obtained from the Cookeina speciosa mushroom were examined under in vitro conditions, and it was observed that (1→3) linked glucans fermented faster.

Understanding the link between their biological effects and structural characteristics is essential for enhancing their application in functional foods and health-promoting products. Accordingly, the aim of this study is to quantify the β-glucan content (on a dry matter basis) in six different mushrooms (Chestnut Mushroom (*Gyroporus castaneus*), Cultivated Mushroom (*Agaricus bisporus*), Shiitake Mushroom (*Lentinula edodes*), Reishi Mushroom (*Ganoderma lucidum*),Truffle Mushroom (Tuber melanosporum), and Oyster Mushroom (*Pleurotus ostreatus*)) and three algae (Ulva spp., Wakame (*Undaria pinnatifida*), and Spirulina (*Arthrospira platensis*)) and to characterize their structural properties.

The studied species were selected based on their commercial availability, frequent use in human nutrition, and previously reported relevance as potential β-glucan sources. The mushroom species represent both widely cultivated (e.g., *Agaricus bisporus*, *Pleurotus*

*ostreatus*) and medicinally valued taxa (e.g., *Ganoderma lucidum*, *Lentinula edodes*). Algal species were chosen to include both marine macroalgae (*Ulva spp.*, *Undaria pinnatifida*) and a microalga (*Arthrospira platensis*) with established nutritional significance.

Although numerous studies have examined β-glucans from individual mushrooms or cereals, comparative analyses involving both fungal and algal matrices under identical extraction and analytical conditions remain limited. The novelty of the present study lies in the unified evaluation of six mushroom species and three algae using the same enzymatic quantification, FTIR characterization, and SEM-based morphological assessment. This approach enables a direct comparison of terrestrial and marine sources in terms of β-glucan content and structural features, thereby providing new insights into their complementary potential for functional food formulations.

## 2. Materials and methods

### 2.1. Extraction of β-glucan

All mushroom samples and algal materials were obtained from local producers in the Aegean region of Türkiye (Izmir province, coordinates approximately 38°25′N, 27°08′E, altitude 25–50 m). Samples were collected during the 2024 harvesting season and transported under refrigerated conditions to the laboratory.

Dried samples were milled to pass through a 250-mesh sieve and stored at 4 °C until analysis. B-glucan content was determined using the Mushroom and Yeast B-Glucan Assay Kit (K-YBGL, Megazyme International, Bray, Ireland), which is based on specific enzymatic hydrolysis followed by spectrophotometric quantification at 510 nm. The method was applied as described by McCleary and Draga (2016).

Alpha-glucan content was measured separately and subtracted from the total glucan to calculate β-glucan levels. All analyses were performed according to the manufacturer's instructions using kit-supplied reagents and controls. (Hettich, Universal 320 R, Germany)

Dried samples were homogenized using a magnetic stirrer for extraction. All chemicals used were of analytical grade (Merck KGaA, Darmstadt, Germany). Glucose concentrations were determined using the Glucose Oxidase/Peroxidase (GOPOD) method included in the kit.

### 2.2. Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR spectral analysis was conducted using a Thermo Scientific NICOLET iS10 spectrophotometer (USA) within the wavenumber range of 400–4000 $cm^{-1}$. The measurements were performed on dried samples to evaluate the characteristic functional groups of mushroom- and algae-derived polysaccharides.

### 2.3. Scanning electron microscope (SEM)

The morphological properties of mushroom and algal samples were examined using a scanning electron microscope (ThermoFisher, QUANTA FEG 250, USA) at the Central Research and Analysis Laboratory of Ege University (Ege MATAL). Prior to imaging, the samples were coated with a conductive layer of gold-palladium. SEM images were captured at a magnification of 10000×.

### 2.4. Statistical analysis

The statistical evaluation of the data was conducted using SPSS software (version 25.0). One-way analysis of variance (ANOVA) was applied to test the significance of differences in glucan content among the samples. When significant differences were found, Duncan's Multiple Range Test was used to identify specific group differences. A significance level of $p<0.05$ was considered statistically meaningful. All results are presented as mean ± standard deviation.

## 3. Results and discussions

### 3.1. Extraction of β-glucan

Significant differences were observed in the glucan content (on a dry matter basis) among the various mushroom and algal samples (Table 1). *Ganoderma lucidum* (Reishi) showed the highest total glucan content at 66.04%, which was statistically greater than that of all other samples ($p < 0.05$), indicating its value as a

potent biomass source for glucan extraction. Approximately two-thirds of the total glucan content in Reishi (43.85%) was composed of β-glucans, highlighting not only its high overall glucan content but also its dominance in the β-glucan fraction. This composition aligns with various biological activities previously reported in the literature, such as immune modulation, enhanced antioxidant capacity, and antitumor effects (Zhu et al. 2015b). For instance, Zhu et al. (2015a) emphasized the abundance of β-(1→3) and β-(1→6) glucosidic linkages in medicinal mushrooms like Reishi, which are known for their strong immunomodulatory properties.

**Table 1.** Total, alpha, and β-glucan contents of the samples (% w/w on dry matter basis)

| Sample | Total Glucan (%) | Alpha-Glucan (%) | β-Glucan (%) | Reported β-Glucan (%) |
|---|---|---|---|---|
| *G. lucidum* | 66.04±3.85f | 22.19±6.06b | 43.85±2.21f | >40 (Xu et al., 2011) |
| *L. edodes* | 26.76±0.6e | 0.94±0.18a | 25.82±0.85e | 29.32(Morales et al., 2018) |
| *T. melanosporum* | 22.90±0.46d | 3.59±0.01a | 19.31±0.45d | 20.27 (Tejedor-Calvo et al., 2020) |
| *P. ostreatus* | 22.83±1.31d | 1.02±0.06a | 21.80±1.25d | 15.15 (Setyawan et al., 2024) |
| *A. bisporus* | 14.63±2.27c | 1.23±0.03a | 13.40±2.30c | 12.22 (Setyawan et al., 2024) |
| *G. castaneus* | 10.94±0.65bc | 1.21±0.11a | 9.73±0.55b | - |
| *U. lactuca* | 9.71±1.20b | 1.53±0.36a | 8.18±1.56b | - |
| *A. platensis* | 7.17±0.50b | 3.77±1.60a | 3.39±2.09a | 13.72 (Markou et al., 2021) |
| *U. pinnatifida* | 2.68±0.65a | 0.49±0.11a | 2.19±0.55a | - |

* Letter differences in the columns for total, alpha, and β-glucan contents indicate statistical group difference

The quantitative ratios of glucan fractions (total, alpha, and beta) play a crucial role in determining the functional and technological application potential of these compounds. Due to their insolubility and viscosity-building capacity, β-glucans are highly desirable in the formulation of functional foods, nutraceuticals, and pharmaceuticals (Zhu et al. 2016). In contrast, alpha-glucans primarily serve as digestible energy sources and generally exhibit limited biological activity (Du et al. 2019; Boulaka et al. 2020). Therefore, the high β-glucan-to-total-glucan ratio of Reishi enhances its value as a raw material for immune-supportive formulations. Moreover, the branching structure, molecular weight, and solubility of β-glucans support their use as stabilizers, gel-forming agents, and thickeners in food technologies.

Similarly, *Lentinula edodes* (25.82%), *Tuber melanosporum* (19.31%), and *Pleurotus ostreatus* (21.80%) also exhibited statistically significant levels of β-glucan content. These samples are thus considered promising candidates for use in functional food formulations. Consistent with these findings, Pérez-Bassart et al. (2023) also reported high biological activity of β-glucans extracted from shiitake mushrooms, emphasizing that the efficiency can vary depending on both the fungal species and the extraction technique.

The yield, purity, and bioactivity of β-glucans are known to be significantly influenced by the extraction method (Burkus and Temelli 1999; Ahmad et al. 2009). Minor modifications in the extraction procedure can affect molecular weight, solubility, viscosity, color, and functional behavior of β-glucans (Burkus and

Temelli 1999; Ahmad et al. 2009). In recent years, enzyme-assisted extraction methods have emerged as more efficient and environmentally friendly alternatives to conventional water or alkaline treatments (Liu et al. 2021). In this study, a combination of acid and enzymatic techniques was employed to preserve cell wall integrity and maintain the biofunctionality of the extracted glucans.

Enzyme-assisted extraction offers distinct advantages, particularly for insoluble polysaccharides such as β-glucans. It allows for high yield, reduced energy consumption, and shorter processing times, which are especially valuable in the food industry (Rosenthal et al. 1996; Nyam et al. 2009; Yin et al. 2011). As reported by Li et al. (2012), enzymatic treatment enhances cell wall breakdown, facilitating better solvent access and improving extraction efficiency. Furthermore, the removal of proteins and starch by enzymatic hydrolysis results in a purer glucan extract (Beer et al.1997a; Beer et al. 1997b; Goudar et al. 2020). B-glucans isolated via enzymatic hydrolysis also tend to possess higher molecular weights and viscosity, which contribute positively to their functional properties (Gamel et al. 2014).

In contrast to the mushrooms, algal samples such as Spirulina platensis (3.39%) and *Undaria pinnatifida* (2.19%) displayed significantly lower levels of β-glucan content. This observation is consistent with the literature, which suggests that macroalgae are generally richer in other bioactive compounds such as soluble dietary fibers, diverse polysaccharides, and polyphenols rather than β-glucans. Nevertheless, it has been reported that β-glucan production in Spirulina can be significantly enhanced under phosphorus-limited culture conditions, indicating that cultivation parameters can influence β-glucan yields even before the extraction stage (Markou et al. 2021). Moreover, studies have shown that combinations of Spirulina and β-glucans may exert synergistic effects. Mokhbatly et al. (2020) demonstrated that co-administration of Spirulina and β-glucans mitigated chlorpyrifos-induced toxicity in Clarias gariepinus, resulting in improved immune responses and reduced oxidative stress. These findings suggest that even sources with inherently low β-glucan content can offer functional benefits when combined strategically with other compounds.

Despite its low glucan content, wakame also presents promising biological activities. Niu et al. (2015) reported that dietary inclusion of 2% wakame significantly improved growth performance, protein retention, and immune response in young Penaeus monodon shrimp. These results highlight the importance of fucoidan and other polysaccharides in wakame that may contribute to its functional potential beyond β-glucan content.

*Ganoderma lucidum* emerges as the most potent β-glucan source suitable for nutraceutical and pharmaceutical applications, other mushroom samples such as shiitake, truffle, and oyster also demonstrate considerable potential (Zhu et al. 2015). On the other hand, algae like Spirulina and wakame, though relatively low in β-glucans, may still offer valuable bioactivities through optimized culture conditions or synergistic formulations.

### 3.2. Fourier transform infrared spectroscopy analysis

The FT-IR spectra, highlighting key functional group vibrations associated with β-glucans and other polysaccharides, are presented in Figure 1.

A broad stretching band associated with hydroxyl (–OH) groups in the polysaccharide structures was observed in the 3600–3000 $cm^{-1}$ region of the spectra, which is the first characteristic region for polysaccharides (Setyawan et al. 2024). Similar spectral regions have been reported in the literature to reflect intra- and intermolecular hydrogen bonding in polysaccharide structures (Synytsya and Novak 2014). This band appeared with marked intensity, particularly in Reishi, Shiitake, Chestnut, Cultivated, and Oyster mushroom samples, indicating the abundance of hydrogen bonds and hydrophilic groups along the glucan chains in these samples (Kačuráková and Wilson 2001). In contrast, the corresponding band in the algal samples Spirulina, Ulva spp., and Wakame was significantly weaker.

Narrow bands in the 3000–2800 $cm^{-1}$ region correspond to stretching vibrations of aliphatic C–H bonds (Setyawan et al. 2024). Reishi, Shiitake, Cultivated, and Oyster mushrooms exhibited the highest absorbance in this region. These bands, attributed to the C–H stretching vibrations of methyl and methylene groups in the glucopyranoside ring, are recognized as key structural markers of β-glucans (Synytsya and Novak 2014; Kačuráková and Wilson 2001).

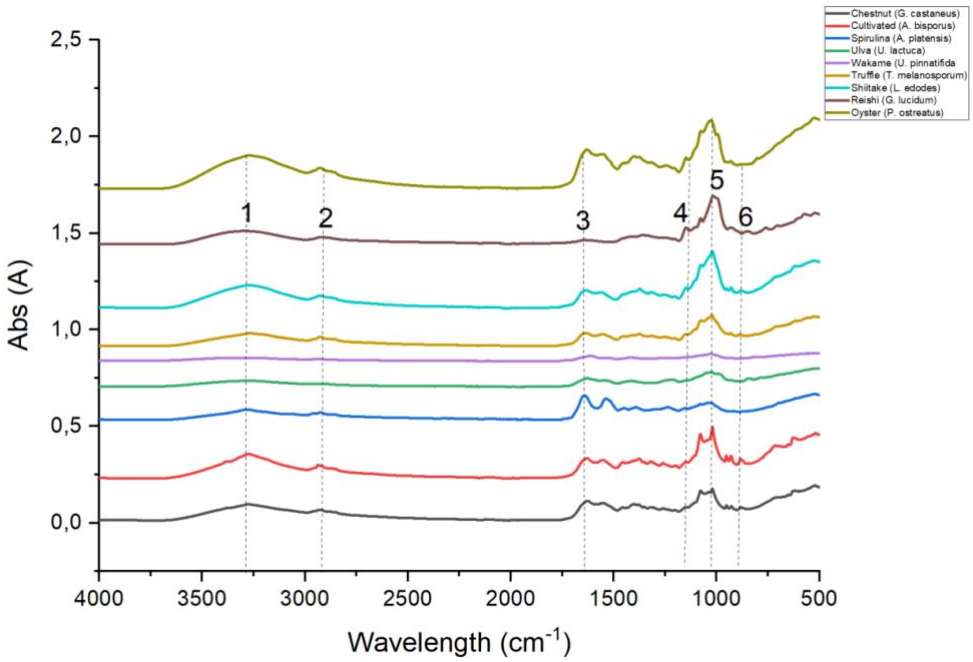


**Figure 1.** FT-IR spectra of the samples

In the spectral region of 1700–1600 $cm^{-1}$, known as the Amide I band, pronounced absorbance peaks were detected in Shiitake, Truffle, Oyster, Cultivated, and Chestnut mushrooms, as well as in Spirulina. This band is associated with the stretching vibrations of carbonyl (C=O) groups and indicates the presence of protein residues or protein–glucan complexes (Setyawan et al. 2024).

The most characteristic region for polysaccharide structures appeared between 1200–1000 $cm^{-1}$. These bands are related to C–O–C and C–C stretching vibrations and are strong indicators of β-glucan presence (Kačuráková and Wilson 2001). Intense absorbance in this region was observed in Reishi, Shiitake, Oyster, and Cultivated mushroom samples. While the band was broader and more diffuse in Reishi, it was sharper and more symmetrical in the Oyster mushroom. This may suggest a coexistence of alpha- and β-glucans in Reishi, whereas in Oyster, the glucan structure is predominantly composed of β-glucans (Synytsya and Novak 2014). Conversely, in Spirulina, Wakame, and Ulva spp., this region showed weak and dispersed bands, indicating a lower prevalence of β-glucan structures and the possible dominance of alternative polysaccharides in these algae.

A band observed near 890 $cm^{-1}$ corresponds to deformation vibrations of β-(1—3) glycosidic linkages. This band was observed in samples such as Reishi, Cultivated, Shiitake, and Chestnut mushrooms and supports the presence of linear β-glucan structures (Setyawan et al. 2024). It was not detected in the algal samples.

### 3.3. Scanning electron microscope

The microstructures of samples examined via scanning electron microscopy (SEM) to assess surface morphology, cell wall integrity,

and porosity as indicators of the potential extractability of cell wall polysaccharides such as β-glucans.

In mushroom samples, the SEM images revealed fibrous, porous, and partially compact cell wall architectures. Notably, *Gyroporus castaneus, Pleurotus ostreatus, Ganoderma lucidum*, and *Lentinula edodes* showed fibrous microstructures and surface deformations that may enhance solvent interaction and facilitate glucan extraction (Rosenthal et al. 1996; Nyam et al. 2009; Yin et al. 2011; Novák et al. 2012). In contrast, *Tuber melanosporum* and *Agaricus bisporus* exhibited denser and more intact cell wall structures, suggesting the need for alkaline or mechanical pre-treatment before effective extraction (Liu et al. 2021a; Novák et al. 2012). The overall structural pattern—mainly consisting of β-(1→3) and β-(1→6) glucans—was consistent with the known cell wall composition of edible mushrooms. *Ganoderma lucidum* particularly exhibited layered and highly fibrous walls, which coincide with its remarkably high β-glucan content (43.85%). This structural observation supports the quantitative data. Similar structural patterns were reported in SEM studies of Saccharomyces cerevisiae, where fibrous, granular, and non-compact formations facilitated the release and formation of β-glucan-rich films (Novák et al. 2012). Sample SEM images of these structures are presented in Figure 2.

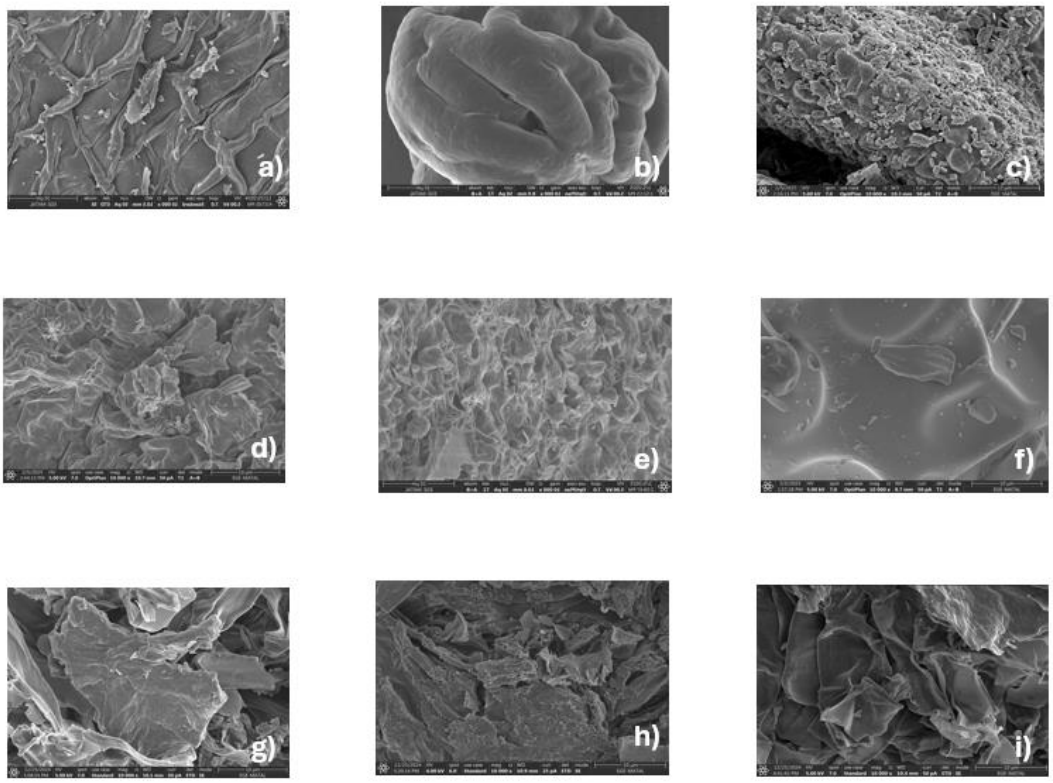


**Figure 2.** SEM images of samples (10000× magnification) of: (a) Ulva spp., (b) Spirulina, (c) Wakame, (d) Truffle mushroom, (e) Shiitake mushroom, (f) Reishi mushroom, (g) Oyster mushroom, (h) Cultivated mushroom, (i) Chestnut mushroom.

The microstructures observed in algal samples (Ulva spp., Wakame, and Spirulina) were distinctly different from those found in mushroom samples. Ulva spp. exhibited layered and scaly structures, suggesting the presence of sulfated, water-soluble polysaccharides such as ulvan in sheet-like formations. In wakame, porous and folded surface morphologies were observed, indicating a heterogeneous distribution of structural polysaccharides like

fucoidan and alginate. The filamentous structure of spirulina was generally preserved, though partial disintegration was evident in some samples. This structural breakdown may facilitate solvent access to intracellular contents, thereby enhancing the extraction efficiency of β-glucan-like polysaccharides (Liu et al. 2021).

Structural polysaccharides in algae tend to be more water-soluble and amenable to extraction, whereas glucans in mushroom cell walls are typically embedded in more compact matrices, leading to lower solubility but higher biological activity. These differences were clearly reflected in the SEM images. The variation in glycosidic linkage types (β-1,3; β-1,4; β-1,6) and branching degrees between algal and fungal glucans also significantly influences both their extraction behavior and functional applications (Kaur and Riar 2020).

One study noted that more homogeneous, spherical, and smaller microstructures increased surface area and improved both biological and functional performance (Anusuya and Sathiyabama 2014). Therefore, SEM imaging can serve as a valuable tool to assess the processability and functional potential of biomass sources rich in β-glucans. Structural criteria such as cell wall integrity, the presence of fibrous structures, porosity levels, and surface morphology are directly related to extraction efficiency (Liu et al. 2021; Novák et al. 2012). Samples with fibrous and porous architectures (e.g., Reishi, Shiitake, Oyster) allow better penetration of solvents or enzymes into the cell wall matrix, thereby yielding higher extraction efficiency (Rosenthal et al. 1996; Nyam et al. 2009; Yin et al. 2011). Conversely, samples with compact and dense cell wall structures (e.g., Truffle, Cultivated mushroom) may require physical or chemical pre-treatment before extraction (Liu et al. 2021).

These morphological traits not only influence process efficiency but also determine the functional properties of the extracted β-glucans. Well-extracted, high-molecular-weight β-glucans typically exhibit greater viscosity, gel-forming ability, and prebiotic effects, making them more desirable for use in functional foods, capsule formulations, and health-supporting products (Du et al. 2019; Boulaka et al. 2020; Zhu et al. 2015). Thus, cell wall-based evaluations provide valuable insights into the industrial processability and product development feasibility of candidate samples. A summary of the SEM-based structural evaluations is presented in Table 2.

**Table 2.** SEM microstructures and cell wall status of the samples

| Sample Type | SEM Structure | Cell Wall Condition | B-Glucan Potential |
|---|---|---|---|
| *Gyroporus castaneus* | Fibrous, porous | Degraded | High |
| *Agaricus bisporus* | Compact | Partially preserved | Medium |
| *Lentinula edodes* | Fibrous, fragmented | Moderately degraded | High |
| *Ganoderma lucidum* | Layered, fibrous | Partially degraded | Very high |
| *Tuber melanosporum* | Compact, dense | Preserved | Medium |
| *Pleurotus ostreatus* | Porous, fibrous | Degraded | High |
| Ulva spp. | Layered, flaky | Layered | Medium–High |
| *Undaria pinnatifida* | Porous, folded | Degraded | Medium |
| *Arthrospira platensis* | Filamentous | Partially fragmented | Medium |

## 4. Conclusions

The results of this study demonstrate that the β-glucan content and structural characteristics of mushrooms and algae vary significantly depending on the biological source. Among the evaluated samples, *Ganoderma lucidum* stands out with its exceptionally high β-glucan yield and structurally fibrous, porous cell wall, confirming its status as a potent raw material for bioactive polysaccharide extraction. *Lentinula edodes* and *Pleurotus ostreatus* also exhibited favorable structural and compositional properties, reinforcing their potential in health-promoting food applications. In contrast, the algae, particularly Spirulina and Wakame, displayed lower β-glucan contents but retained distinct morphological and chemical features that may contribute to complementary bioactivities when used in combination with fungal glucans. FT-IR and SEM analyses proved to be essential in distinguishing β-glucan presence and assessing extraction potential, providing a reliable framework for selecting optimal biomass sources. Future studies may focus on optimizing cultivation and extraction conditions to enhance the bioavailability and application scope of β-glucans from both terrestrial and marine sources. A limitation of this study is the absence of in vitro or in vivo biological validation assays; therefore, future work should include immunomodulatory, antioxidant, and prebiotic activity tests to further substantiate the functional relevance of the characterized β-glucans.

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