β-Glucan Extraction from Mycelium in Spent Mushroom Substrate of *Pleurotus ostreatus* and *Schizophyllum commune*

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**Abstract.** Spent mushroom substrate (SMS) is the biomass waste produced from the production of mushroom which generating disposal problems. To overcome the problem the extraction of bioactive compound such as β-glucan from the waste SMS could solve the problem and can also increase its added value. β-glucan appears to be promising for aiding in the cure of tumorous disease and help to reduce the cholesterol levels in blood. In this study, β-glucan was extracted and compared from two different commercial mushroom (*Pleurotus ostreatus* and *Schizophyllum commune*) from its fruiting body and mycelium on solid waste SMS using chemical extraction methods. The characteristics of physical structure, functional group and properties of extracted β-glucan was investigated. Here, Fourier-transform infrared (FTIR) and screening electron microscope (SEM) were used to identify and evaluated the structural conformations of β-glucan and physical structure. The functional properties, swelling power, viscosity and fat binding capacity were analyzed. Based on results, mycelia of *Pleurotus ostreatus* shown highest swelling power (11.74 g/g) and fat binding capacity (12.09 g oil/g sample) while, mycelia of *Schizophyllum commune* shown the highest viscosity (11.85 cP). Since the value for all functional properties shown the highest value on mycelium compare to fruiting body, thus it is strength that β-glucan extraction from mycelium solid waste has high novel properties compare with mushroom fruiting body.

**1. Introduction**

World creation of developed, palatable mushroom has ascended from 1 billion kg in 1978 to 34 billion kg in 2013 and was esteemed at roughly USD 34 billion [1]. The situation is driven by high demand for medicinal, nutraceutical and cosmetic products using mushroom as their raw materials and expected to increase in the future. Moreover, the market for mushroom based products like beverages and snacks are also predicted to increase tremendously [2]. Approximately 5 kg of spent mushroom substrate (SMS) was generated from the production of 1 kg of mushroom [5, 6]. The current method of disposal of SMS are by burning, spreading on land, burying, composting with animal manure and landfiling had generated adverse effect to the environments since water vapour and carbon dioxide were generated from the burning of SMS as primary products and contributes directly to the carbon dioxide greenhouse
Effect which affecting human health [7]. These disposal methods create adverse effect to the environments since water vapour and carbon dioxide were generated from the burning of SMS as primary products. It contributes directly to the carbon dioxide greenhouse effect [8] and affecting human health [9]. Thus, various methods of recycling SMS were carried out such as animal feeding, energy feedstock [7] and extraction of bioactive compound [10]. Mushroom has been recognised as a natural source for the development of nutraceuticals and medicinal products [3], due to one of the bioactive compounds that have useful impacts on human wellbeing which is β-glucans [4].

One of the valuable bioactive compounds extracted from SMS is β-glucan that appear to be promising for aiding in the cure of tumorous disease and helps to reduce the cholesterol levels in blood [11]. The β-glucan could be obtaining from either fruiting body or mycelium part in the mushroom. There are various method to extract β-glucan from mushroom such as using aqueous ethanol and enzymes [12], water distillation at 100°C in the ratio weight to volume of 1:10 [13], KOH-fraction, HCl-fraction, NaOH-fraction [14], enzymatic method [15], and solubilising in hot water or alkaline solution [16]. Thus, the aim of this report was characterized the properties of β-glucan on fruiting body and mycelium sawdust SMS in two different species that cultivated locally in Malaysia. Previously, there were many report on β-glucan extraction and characterization however, there is lack information on extraction of split grill mushroom. Here, Pleurotus ostreatus and Schizophyllum commune, or commonly known as oyster mushroom and split gill mushroom respectively were used in the investigation, using a chemical extraction method. Next, the physical properties and the functional group of the extracted β-glucan from mycelium with the fruiting bodies of the mushroom was compared.

2. Materials and methods

2.1. Material

Pleurotus ostreatus and Schizophyllum commune were harvested and collected from Uniciti Alam, UniMAP and Institute of Sustainable Technology (INSAT), UniMAP respectively. All of fruiting body mushroom and mycelium from both species were freeze-dried (LaboGene, Scandinavian) overnight before an extraction. NaOH and HCl were obtained from Sigma-Aldrich. Soy oil was purchased from Somca Trading Malaysia.

2.2. Extraction of β-glucan

7 g of the freeze-dried fruiting body mushroom or mycelium undergoing a 20 min extraction process under a constant stirring with 120 mL NaOH at 60°C in a round bottom flask as in Figure 1. The suspension was filtered, and the filter cake was washed with distilled water. The filtrate was collected and neutralized with 6 mol/L HCl. The neutralized filtrate was dried at room temperature [14].

Figure 1. Apparatus setup for the extraction of β-glucan.
2.3. Evaluation of the characteristics of the extracted total β-glucan

2.3.1. Physical structure of the extracted total β-glucan. The physical structure of the mycelium of mushroom was determined using Scanning Electron Microscope (SEM) from JEOL JSM-7001F, Germany. A sputter coater was used to coat a thin layer of platinum on the sample. Then, the coated sample was mounted on the SEM aluminium stubs with double sided tape. After coating vertically using platinum, the sample was photographed at an accelerator potential of 5 kV. The images samples of 5000x magnification were captured for each extracted β-glucan sample.

2.3.2. Analysis of FTIR of the extracted total β-glucan. ATR-FTIR spectroscopy was used to identify the functional group of the extracted β-glucan at room temperature in the wavelength region between 4,000 and 400 cm⁻¹. The FTIR spectra of extracted β-glucan was recorded on Perkin Elmer Spectrum 100 series FTIR spectrometer, USA.

2.4. Evaluation of the functional properties of the extracted total β-glucan

2.4.1. Swelling power. The swelling power of the total β-glucan extracted was determined referring to the published journal [18]. A mixture of 5 mL distilled water and 0.15 g total β-glucan were placed in a shaking water bath at 70°C for 10 min before being transferred to a boiling water bath for 10 min. The tubes were cooled for 5 min with tap water and were centrifuged at 1336 rpm for 4 min. The swelling power was determined using Equation (1).

\[
\text{Swelling power} = \frac{W}{D} \tag{1}
\]

Where, W is weight of wet sediment (g) and D is weight of dry sample (g)

2.4.2. Viscosity. The viscosity of the total β-glucan extracted was determined using Brookfield DV2T, USA. Viscosity of the extracted β-glucan (1% w/v) was measured at room temperature using Brookfield viscometer. Here, 0.01 g of β-glucan was immersed in 100 mL of distilled water in the 250 mL beaker. Then, the mixture was measured by using RV03 spindle. The analysis was operated for 1 min at the speed of 180 rpm.

2.4.3. Fat binding capacity. 5 mL of soy oil was dispersed in 0.1 g total β-glucan. The mixture was placed for 1 hour in ambient room temperature and agitated every 15 min on a vortex mixer. The mixture was centrifuged at 1258 rpm for 20 min. The supernatant was drained, and the residue was weighed. The fat binding capacity was determined using Equation (2).

\[
\text{Fat binding capacity} = \frac{W}{D} \tag{2}
\]

Where, W is weight of wet sediment (g oil) and D is weight of dry sample (g sample).

3. Results and discussion

3.1. Characteristics of the extracted β-glucan

Micrographs of the four samples of β-glucan had been obtained using SEM imaging as shown in Figure 2. Figure 2 (a) and (b) shows the mushroom samples of Pleurotus and Schizophyllum, while Figure 2 (c) and (d) shows the mycelia samples of Pleurotus and Schizophyllum.
Figure 2. SEM images of (a) β-glucan Pleurotus (fruiting body), (b) β-glucan Schizophyllum (fruiting body), (c) β-glucan Pleurotus (mycelia) and (d) β-glucan Schizophyllum (mycelia)

All four samples showed a typical creases and furrows structure. However, rough surface of β-glucan from Pleurotus on both fruiting body and mycelia was observed and its obtained on the creases part compare to the surface of β-glucan from Schizophyllum. Thus, it was shown that the structure of β-glucan from Pleurotus were more roughness compare to Schizophyllum.

3.2. FTIR analysis of the extracted β-glucan

The functional group of the extracted β-glucan was evaluated using ATR-FTIR, to confirm the presence of β-glucan on the extracted polysaccharide of both mushroom and mycelia. The ATR-FTIR spectra of four samples of β-glucan are shown in Figure 3 (a) to (d). Figure 3 (a) and (b) show the ATR-FTIR spectra of mushroom samples of Pleurotus and Schizophyllum respectively, while Figure 3 (c) and (d) show the ATR-FTIR spectra of mycelia samples of Pleurotus and Schizophyllum respectively. This method is sensitive to the position and anomic configuration of the glycosidic linkage in β-glucan [17]. All four sample of β-glucan shown the presence of hydroxyl (–OH) group from the glucose structure in sugar ring, in the range of wavelength between 3600 – 3200 cm⁻¹. Furthermore, all four sample shows the presence of methyl (–CH₃) group at the wavelength range of 2500 – 3000 cm⁻¹. In Figure 3 (c) and (d) for both mycelia Pleurotus and Schizophyllum, the peak for methyl group was strong as compared to other β-glucan samples on fruit body mushroom but very strong and high peak obviously was obtained on Figure 3 (d).

In the range of wavelength between 1745 – 1762 cm⁻¹, the spectra shown the presence of carbonyl (C=O) group. In this range, the β-glucan sample of mushroom Pleurotus in Figure 3 (a) shows a slightly intense peak as compared to mycelia Pleurotus in Figure 3 (c), while the β-glucan sample of mushroom Pleurotus shows an intense peak as compared to mycelia Pleurotus. A characteristic β-linkage was shown by all four sample of β-glucan that lies in the wavelength range of 884 – 892 cm⁻¹.
However, in Figure 3 (a) for mushroom *Pleurotus*, the peak was not as clear as in Figure 3 (c) for mycelia *Pleurotus* which indicates that it probably has less β-glucan compared to mycelia *Pleurotus* that shows a clear peak. β-glucan content depends on the degree of fruiting bodies maturity, the higher the β-glucan content, the higher the degree of fruiting bodies maturity [11]. This might be the reason for the characteristics β-linkage in mushroom *Pleurotus* is not as intense as in mycelia *Pleurotus*. In this spectrum, a presence of protein was detected lies in the range of 1560 – 1575 cm\(^{-1}\), but the type of protein is unknown as there were no additional information regarding the presence of protein in the structure of β-glucan.

![ATR-FTIR spectra](image)

**Figure 3.** ATR-FTIR spectra of (a) *Pleurotus* (mushroom) or fruiting body, (b) *Schizophyllum* (mushroom) or fruiting body, (c) *Pleurotus* (mycelia) and (d) *Schizophyllum* (mycelia)
3.3. Functional properties of extracted β-glucan

3.3.1. Swelling power. Swelling power indicates the water holding capacity of β-glucan that is commonly used to indicate the differences between various types of β-glucan samples [19]. Table 1 shows the result for swelling power of mushroom and mycelia in Pleurotus and Schizophyllum.

| Species                | Swelling power (g/g) |
|------------------------|----------------------|
| Pleurotus (mycelia)    | 11.74                |
| Pleurotus (fruiting body) | 8.52                |
| Schizophyllum (mycelia) | 4.27                 |
| Schizophyllum (fruiting body) | 5.25                |

The swelling power of between these two species shown that the Pleurotus species higher as compared to Schizophyllum species and thus, it shown that the β-glucan of Pleurotus species had water holding capacity. For the Pleurotus species, the mycelia shown the highest swelling power as compared to the fruiting body. In contradict, β-glucan from the fruiting body mushroom of Schizophyllum species had the highest swelling power as compared to the mycelia. This finding was supported by the ATR-FTIR spectra on Figure 3 (d), where a strong peak of –CH₂ in mycelia Schizophyllum was obtained. This indicates that in mycelia Schizophyllum, it absorbing less water compared to fruiting body Schizophyllum due to its higher non-polar characteristic. Previous journal reported that the swelling power of Coprinus (fruiting body) and Agaricus (fruiting body) were 4.49 g/g and 3.45 g/g respectively [17].

3.3.2. Viscosity. Viscosity is an important property of food that affects the mouth feel and texture of the fluid [20]. Table 2 shows the results for viscosity of fruiting body and mycelia in Pleurotus and Schizophyllum.

| Species                | Viscosity (cP) |
|------------------------|----------------|
| Pleurotus (mycelia)    | 10.93          |
| Pleurotus (fruiting body) | 10.56          |
| Schizophyllum (mycelia) | 11.85          |
| Schizophyllum (fruiting body) | 10.56          |

For the Pleurotus species, the viscosity of β-glucan for mycelia were higher as compare to its fruiting body. This statement supports the ATR-FTIR spectra in Figure 3 (c) and (a), that shows the peak of characteristic β-linkage are strong in mycelia compared to the fruiting body respectively. Similar effect was also observed by Schizophyllum species where the viscosity of β-glucan for mycelia was higher as compared to its fruiting body.

Contradicting to result observed by [17] where, high viscosity of Pleurotus (mushroom) obtained at 178.56 cP compare to this experiment. The situation can be explained due to the difference in the type of spindle used during the measurement, in which a flow resistance change with speed and size of the spindle [21]. Previous study shown that a lower viscosity of β-glucan from shiitake as compare to
oats β-glucan, however here the low viscosity on β-glucan derived from fruiting body is more advantageous in lowering blood cholesterol in the gastrointestinal tract [22].

3.3.3. Fat binding capacity. Fat binding capacity measures the amount of oil absorbed by weight of β-glucan and this property is beneficial in fat and flavour retention and texture [23]. They also state that protein source, size and concentration, number of polar amino acids, processing methods and protein-lipid interactions affects the fat binding capacity. The fat binding capacity is an important feature that offers a proper mouth feel [17]. Fat binding capacity evaluates the dietary fibres ability to avoid fat loss during food processing and minimize serum cholesterol levels by binding fat in the human digestion system [24]. Table 3 shows the results for fat binding capacity of fruiting body and mycelia in Pleurotus and Schizophyllum.

| Species                      | Fat binding capacity (g oil/g sample) |
|------------------------------|--------------------------------------|
| Pleurotus (mycelia)          | 12.09                                |
| Pleurotus (fruiting body)    | 3.96                                 |
| Schizophyllum (mycelia)      | 5.98                                 |
| Schizophyllum (fruiting body)| 2.99                                 |

The fat binding capacity for mycelia on both Pleurotus and Schizophyllum shows the highest value as compared to its fruiting body mushroom part. Similar result obtained by [17] for fat binding capacity as compare to our results where they reported that the fat binding capacity of Coprinus (fruiting body) and Agaricus (fruiting body) 6.65 g oil/g and 5.38 g oil/g respectively. However, no studies have been conducted on mycelia from their previous studies. The results were supported by the FTIR spectrum where both mycelia in Pleurotus and Schizophyllum contain high methyl group. The higher methyl group content is more hydrophobic in nature that can bind strongly to oil compound thus, increasing the fat binding capacity compare to fruiting body of both mushroom species.

4. Conclusion
In conclusion, β-glucan were successfully extracted from various samples using chemical extraction method. The physical structure of β-glucan for Pleurotus species has a rough surface as compared to Schizophyllum species. For the functional group of β-glucan, a characteristics β-linkages was shown by all four sample that lies in the wavelength range between 884 – 892 cm⁻¹. The strong peak for characteristics β-linkages showed in mushroom Schizophyllum compare to Pleurotus. The functional property shown that the highest swelling power and fat binding capacity on mycelia Pleurotus obtained in 11.74 g/g and 12.09 g oil/g sample respectively compare to mycelia Schizophyllum. However, mycelia Schizophyllum shows the highest viscosity which was 11.85 cP. Since the value for swelling power, viscosity and fat binding capacity in mycelia are higher as compared to its fruiting body, it is an advantage for the β-glucan extracted from mycelia had novel properties compared to the mushroom body fruits.

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