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Short Communication

Differential scanning calorimetric analysis of fruit-body and mycelium of *Lentinula edodes*

Sho-ichi Tsujiyama

Department of Agriculture, Kyoto Prefectural University, Shimogamo-nakaragi-cho, Sakyo-ku, Kyoto 606–8522, Japan

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Differential scanning calorimetric (DSC) analysis was applied to the fruit-body (pileus and stipe) and mycelium of shiitake mushroom (*Lentinula edodes*). Thermograms of each sample indicated distinctive patterns. However, chemical and infrared (IR) spectroscopic analyses showed that the compositions of pileus and stipe were similar to each other and different from that of mycelium. Because the DSC thermogram depends not only on chemical composition but also on physical properties such as density, the result of DSC analysis was assumed to indicate a difference in the state of cell wall between pileus and stipe.

Key Words——chemical composition; differential scanning calorimetry (DSC); *Lentinula edodes*; shiitake mushroom.

The chemical compositions of edible mushrooms have been investigated from the viewpoint of food science (Sawada, 1965; Kurasawa et al., 1982; Yoshida et al., 1982, 1986b). For example, chemical changes in fruit-body and mycelium during development of shiitake mushroom have been examined (Yoshida et al., 1986a, 1987; Aoyagi et al., 1993). However, chemical analysis is limited by the difficulty of the procedures involved. Furthermore, the differences between sample data are frequently very small, even though they are significant. Therefore, a convenient procedure is needed for determining differences in fungal cell composition, which would be useful for evaluating the degree of maturity of mycelium on sawdust and bed-log cultivations.

I have applied differential scanning calorimetry (DSC) for analyzing the fungal cell wall. Because thermal analyses, such as DSC and differential thermal analysis (DTA), have been used for the detection of plant materials in peat and the estimation of differences in chemical composition in higher plants (Mitchell, 1957, 1969; Mitchell and Knight, 1965; Stewart et al., 1966; Reh et al., 1986), these are expected to be useful for the analysis of the fungal cell wall. In this study, DSC analysis of the fungal cell wall was compared with chemical and other instrumental analyses.

The test fungus, *Lentinula edodes* (Berk.) Peg., was a stock strain in the Kyoto Prefectural University Forestry Collection (KPUF 0195). Fruit-bodies of *L. edodes* were cultivated in 200-ml Erlenmeyer flasks with 50 ml of PG medium (Kawamura et al., 1983), and harvested when gills became fully exposed but spores did not fall. After removal of peel, fruit-bodies were separated into pileus and stipe. Both were lyophilized, and then milled. Mycelium was incubated in SMY medium (sucrose, 1%; malt extract, 1%; and yeast extract, 0.4%) at 28°C for 2 wk, collected by filtration with a cloth filter, washed with distilled water, lyophilized, and then milled. The milled samples were extracted successively with diethyl ether, 90% aqueous acetone, 70% aqueous methanol and distilled water. After drying, these powder samples were used for the following analyses.

One mg of dried samples was analyzed by use of a differential scanning calorimeter (Seiko Industry; DSC-220) from 50 to 600°C in an ambient atmosphere at a constant heating rate of 10°C/min. Figure 1 shows DSC charts of three samples. These similarly showed no remarkable peaks below 200°C and the major peak at around 330°C (pileus, stipe and mycelium; 331.4, 334.2 and 336.1°C, respectively), of which that of the mycelium sample was highest. However, the patterns of each sample were different at over 400°C. Peaks of pileus, stipe, and mycelium were observed at 484.6, 473.2, and 506.7°C, respectively. Additionally, a peak at 562.8°C was observed in the stipe thermogram, and a shoulder at 270.0°C in the mycelium one. The peaks at around 330°C were mainly due to carbohydrates (Reh et al., 1986; Tsujiyama, 1996), which are major constituents of cell wall. Other peaks have not been assigned. DSC analysis thus revealed differences between these samples.

Chemical composition was analyzed as follows; Cell wall samples were hydrolyzed according to Saeman et al. (1954). Neutral sugars were converted to alditol acetates and analyzed by use of a gas-liquid chromatography Shimadzu GC-12A equipped with a flame ionization detector on a column of OV-225 (2.6 mm × 2.1 m: GL Science). Total carbohydrate content was determined by the phenol-sulfuric acid method. Uronic acid

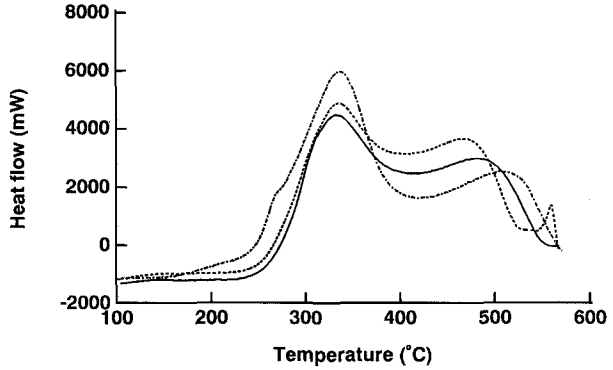

Fig. 1. Thermograms of fungal cell wall samples of *Lentinula edodes*.
——: Pileus, ·····: Stipe, -·-·-: Mycelium.

Table 1. Chemical compositions of cell walls of *Lentinula edodes*.

	Pileus	Stipe	Mycelium (%)
Total sugar	68.7	68.2	60.0
Glucose	93.4	95.4	83.9
Mannose	3.2	2.9	7.9
Galactose	2.1	1.2	6.9
Xylose	1.3	0.2	0.5
Arabinose	0.0	0.3	0.9
Uronic acid	3.4	3.3	2.3
Protein	19.2	15.7	25.8
Total	91.3	87.2	88.1

content was estimated by the carbazole method according to Bitter and Muir (1962) using glucuronic acid as a standard. Protein content was calculated from nitrogen content, which was estimated by the micro-Kjeldahl method.

Chemical compositions of cell walls are shown in Table 1. Carbohydrates were major components of each sample, and glucose was detected in the largest amount, indicating that glucan is the main component. Uronic acids were detected in pileus and stipe more than in mycelium. Mycelium had lower contents of carbohydrates than others, but higher content of protein. The chemical compositions of pileus and stipe were similar except for protein content, though their thermograms were different.

Infrared (IR) spectra of cell wall samples were measured using KBr discs in a Shimadzu FTIR-8200PC. In each spectrum (Fig. 2), remarkable peaks were observed at 1,650 and 1,550 cm^{-1}, which are due to amide I and II, respectively, in protein and chitin. Intensities of these peaks were stronger in the mycelium sample than the others, probably because of the larger content of protein, as shown in Table 1. The peak at 3,200–3,600 cm^{-1} are attributable to hydroxyl groups of carbohydrates, the main components of cell walls. In this range two peaks were observed in pileus and stipe samples, while the mycelium sample gave one broad peak. IR spectra of pileus and stipe were similar, especially in the ranges of around 3,500–3,300 and 1,200–1,000 cm^{-1}, indicating the similarity of their chemical compositions. Therefore, IR spectra reflected the result derived from chemical analysis.

UV analysis was used for acid hydrolysates of cell

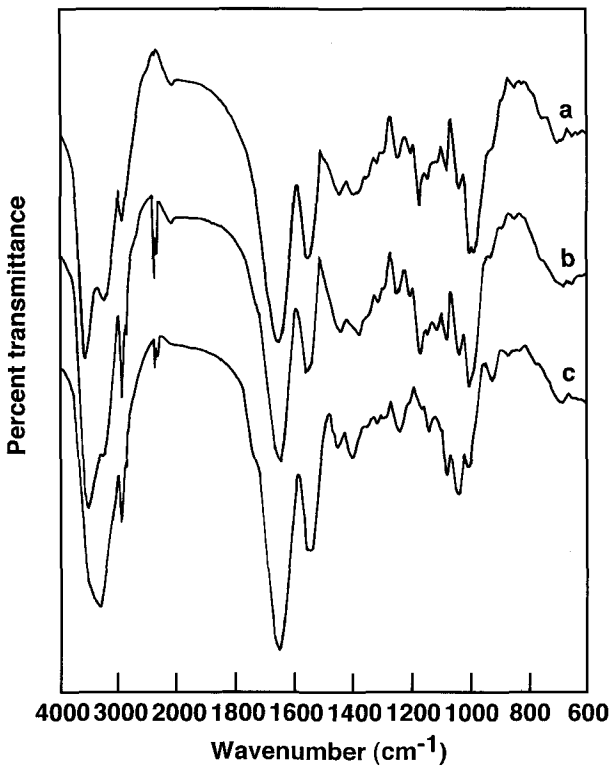

Fig. 2. Infrared (IR) spectra of fungal cell wall samples of *Lentinula edodes*.
a: Pileus, **b**: Stipe, **c**: Mycelium.

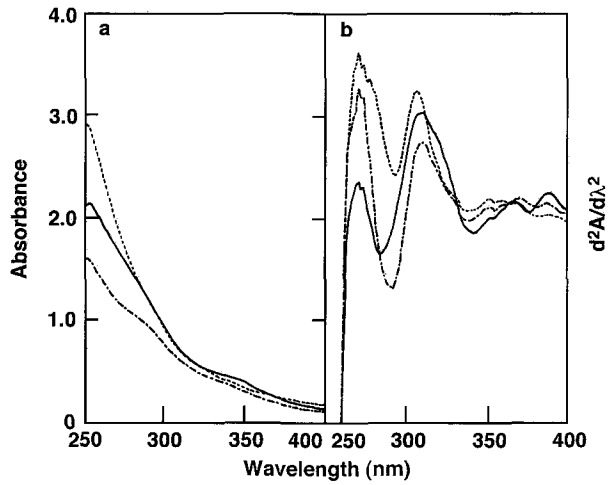

Fig. 3. Ultraviolet (UV) spectra of fungal cell wall samples of *Lentinula edodes*. (Sample concentration: 1 g/l).
a: UV spectra and **b**: Second derivative (2d) spectra.
——: Pileus, ·····: Stipe, -·-·-: Mycelium.

walls of *Mucor rouxii* (Calmette) Wehmer (Bartnicki-Garcia and Nickerson, 1962), but by-products of hydrolysis might interfere with the absorption of cell walls as chromogens. Thus, solubilization procedure with acetyl bromide (Iiyama and Wallis, 1988) was introduced in this study. Solubilization of fungal cell walls was complete after 1 h, and the UV absorbance spectra shown in Fig. 3a were obtained. Each sample showed UV absorption in the range of 250–400 nm. The components detected in this UV range are protein, nucleic acid, melanines, and other pigments. The stipe sample had the strongest UV absorption, suggesting that it has larger amounts of chromogens than other samples or contains the chromogens with high absorptivity. In order to estimate the difference of chromogens, second derivative absorbance spectra (2d-spectra) are shown in Fig. 3b. The 2d-spectra of pileus involved many peaks, indicating the different kinds of chromogens are present. Major peaks were observed at the similar wavelength (pileus; 293.7 and 337.0 nm, stipe; 285.6 and 340.3 nm, mycelium; 291.5 and 337.0 nm), which are probably due to protein, nucleic acids and other aromatic pigments. In these spectra, peak positions of pileus and mycelium were similar, though their intensities were different.

Chemical data on fungal cell walls gave valuable information, which revealed a slight difference between pileus and stipe. Instrumental analysis such as a IR spectroscopy has been used as a convenient method (Sangar and Dugan, 1973; Prieto et al., 1995). In practice, IR data can only support the chemical analysis, as shown above, because it depends on the chemical structures and compositions. UV analysis revealed differences that cannot be observed by chemical and IR analyses, although it could detect only UV-absorbing compounds, not main components such as carbohydrates. On the other hand, DSC could detect differences in fungal cell walls between pileus and stipe, which have similar chemical compositions. The DSC analysis employed in this study is based on the combustion reaction, so the DSC chart depends on the chemical composition and the physical properties of samples such as density; different chemical compositions result in different combustion temperatures, but even with the same chemical composition, a higher density reduced heat conductivity, resulting in the appearance of a peak in the higher temperature range. The difference between pileus and stipe is probably due not to a difference in chemical composition but to a difference in density caused by a difference in the entanglement of macromolecules. Therefore, DSC analysis is expected to reveal differences of state of cell walls, and should be useful for estimating changes in mycelium in the process of differentiation and maturity.

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