Functional group, transversal optic and longitudinal optic analysis using kramer-kronig method of *mycellium* and *oyster* mushroom in 35, 40, and 45 days

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Abstract. *Pleurotus ostreatus*, known as the white oyster mushroom, has higher nutrients than other mushrooms. The purpose of this research is to analyze the result of FTIR spectrum mycelium and oyster mushroom based on the open time of *baglog* after incubation time. Based on FTIR result, function groups of molecules are C-O, C=O, C-H, and O-H in the mycelium and oyster mushrooms. The molecule C-O, C=O, and C-H indicate carbohydrates and O-H molecule suggests water. O-H molecule in the 40th day has low transmittance. It shows that water content in the 40th is more than the 35th days. The value of the absorption energy constant in oyster mushroom is higher than mycelium. The value of spring constant in oyster mushroom is higher than mycelium by the harmonic stretching principle and unharmonic. Based on FTIR spectral at wavenumber range 400–1200 cm^{-1}, Transversal Optic (TO) and Longitudinal Optic (LO) values were obtained using the Kramer-Kronig method.

1 Introduction

Oyster mushrooms (*Pleurotus ostreatus*) are one of the high-level mushrooms that can be easily cultivated and have a wide adaptability to their environment. Oyster mushrooms contain exopolysaccharide compounds that possess antimicrobial, antitumor, anti-inflammatory, and antioxidant properties [1].

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In general, there are three factors that influence the growth of oyster mushrooms, namely (i) temperature, (ii) composition of the growing medium, and (iii) humidity. White oyster mushrooms are suitable for growth at the temperature of 25-27 °C. When these three factors are not met, the production yield of oyster mushrooms will be low [2]. In addition, the techniques used in cultivation also greatly affect the production yield of oyster mushrooms. A common issue is that farmers still struggle to determine the right age to open the planting medium (*baglog*) to achieve optimal results. This research aims to analyze the effect of the opening time after the incubation period of the *baglog* on the FTIR values of the mushrooms and mycelium. Furthermore, it also aims to analyze the effect of that time on the optical properties, as seen from the values of Transversal Optics (TO) and Longitudinal Optics (LO).

2 Experimental method

Composting is the process of mixing all the materials for the growing medium of oyster mushrooms. The required materials consist of 100 kg of wood powder, 1% (1 kg) of corn flour, 2% (2 kg) of agricultural lime, and 18% (18 kg) of bran. *Sengon* wood powder is very good for the growth of oyster mushrooms because it is a substrate material that contains high lignocellulose [3]. The purpose of composting is to degrade the organic materials found in wood powder into simpler compounds, making them easier for the oyster mushroom mycelium to digest. The minimum time for composting is 24 hours [4].

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2.2 *Baglog*

The planting medium (*baglog*) is created by placing the composted mushroom growing medium into plastic measuring (17 x 35 x 0.3) cm. Subsequently, the mushroom growing medium is compacted until its mass reaches 1 kg.

2.3 Sterilization

The purpose of sterilization is to deactivate microbes that will inhibit the growth of oyster mushrooms to be cultivated [5-6]. Sterilization is carried out using a drum. The sterilization time that yields the best harvest is at a steaming duration of 6 hours at a temperature of 220 °C. The amount of water used in the drum is 33 liters. The sterilization process is conducted with two treatments, namely (i) using a sleeve and (ii) without a sleeve, as shown in Figure 1.

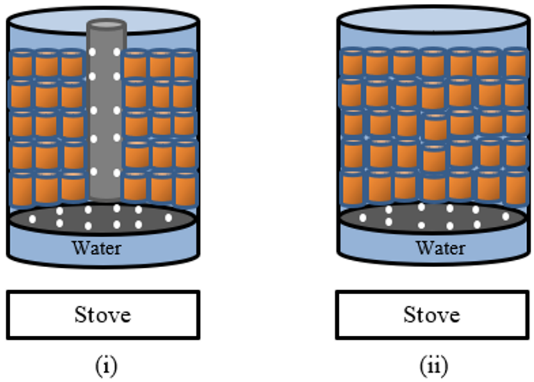

Fig. 1. Design (i) with sleeve and (ii) without sleeve

2.4 Cooling, inoculation, and incubation

The cooling process aims to lower the temperature after the *baglog* is sterilized, so that the cultivated mushrooms do not die during the seeding phase. Cooling is carried out for 24 hours. After that, the *baglog* is ready for inoculation (the planting of mushroom seeds into the *baglog*). The inoculation process must be conducted in a sterile room. The seeds used are mycelium that grows on round corn media. Round corn is utilized as a source of protein. Incubation refers to the storage of the planting media (*baglog*) that has been inoculated in a room with a temperature of (25-28)°C. Inoculation continues until all *baglogs* are filled with white mycelium, which usually takes 30-35 days. The spread of mycelium in the *baglog* significantly affects the formation of oyster mushroom fruit bodies. Once the incubation period is complete, the *baglog* is ready to be opened for the formation of mushroom fruit bodies. The opening time is varied, specifically on the 35th, 40th, and 45th days. After that, the fruit bodies appear, and the mushrooms are ready to be harvested.

2.5 Characterization of the sample using Fourier Transform Infrared (FTIR)

The characterized mushrooms and mycelium must first be dried using a furnace at a temperature of 55 -110 °C until the samples reach a constant weight [7-8]. Subsequently, the sample is ground using a mortar until it becomes a powder. This powder is characterized by FTIR in the wavelength range of (400 – 4000) cm^{-1}, aimed at determining the content of compounds, types of bonds, and optical properties of the mycelium and oyster mushrooms as indicated by the LO and TO value patterns.

3 Results and discussion

3.1 Comparison of harvest results by varying the opening time of incubation

Incubation is the waiting period until the *baglog* is ready for harvesting. After the incubation period is completed, the *baglog* is ready to be opened by varying the incubation opening time, which will result in the emergence of oyster mushroom fruit bodies. The opening times for the *baglog* are at 35 days, 40 days, and 45 days. It can be observed in Table 1 and Table 2 that the most optimal *baglog* opening time is at 40 days, as indicated by the frequency of harvests and the total mass of the harvest being relatively higher compared to the opening at 35 days and 45 days.

At the 35-day opening, the mycelium in the *baglog* is not yet strong enough and still requires time to form fruit bodies, whereas the *baglog* has already been opened and forced to produce fruit bodies, resulting in suboptimal production. At the age of 45 days, the mycelium in the *baglog* is already too old and much of its nutrients have been lost, due to the *baglog* being closed for too long, causing the mycelium to accumulate inside the *baglog* and clump together like crust. Meanwhile, at the age of 40 days, the mycelium in the *baglog* is mature, with sufficient water content and nutrients, and is ready to become fruiting bodies, resulting in optimal harvest yields.

Table 1. Harvest yield data for open incubation time for treatments using sleeves

Open incubation time	The number of *baglogs* growing	The number of contaminated *baglog*	Total mushroom mass (gr)
35	30	4	3450
40	29	5	3525
45	29	5	875
Total	88	14	7850

Table 2. Harvest yield data for open incubation time for treatments without sleeves.

Open incubation time	The number of *baglog*s growing	The number of contaminated *baglog*	Total mushroom mass (gr)
35	16	18	1000
40	16	18	1450
45	16	18	1175
Total	48	54	3625

Based on the results obtained in tables 1 and 2, it is also indicated that the planting medium (*baglog*) with sleeves has a higher total harvest value than that without using sleeves, as the number of contaminated *baglog*s is also lower when using *baglog*s.

3.2 Comparison of harvest results by varying the opening time of incubation

The characterization results of the mycelium and white oyster mushrooms using FTIR revealed the molecular functional groups O-H, C-H, C=O, and C-O. The O-H molecular functional group indicates that the mycelium and oyster mushrooms contain water. Meanwhile, the C-H, C-O, and C-H molecular functional groups indicate that the mycelium and oyster mushrooms contain carbohydrates. When the vibration frequency of a specific sample matches the frequency of infrared radiation directly affecting the molecule, the molecule absorbs the radiation and produces peaks that are analyzed for vibrational analysis, anharmonic constants, and molecular bond force constants, assuming a stretching process. Molecules that appear with only one peak are analyzed under the harmonic principle, while when similar molecules appear with two or more peaks, they will be analyzed under the anharmonic principle.

The mycelium characterized using FTIR under treatments with or without sleeve does not show much difference at ages 35 days, 40 days, and 45 days. The functional groups formed are the same, but their transmittance values differ. It is observed that the O-H functional group at 35 days shows a higher transmittance value compared to 40 and 45 days. A high transmittance value indicates that the water content at 35 days is lower compared to 40 and 45 days. At 45 days, the low transmittance value indicates a high water content. Meanwhile, at 40 days, the water content is optimal for fruit body formation. The area under the curve indicates the amount of energy absorption received by the sample. Figures 2 show that the transmittance results decrease as the age of the mycelium and mushroom in the *baglog* increases.

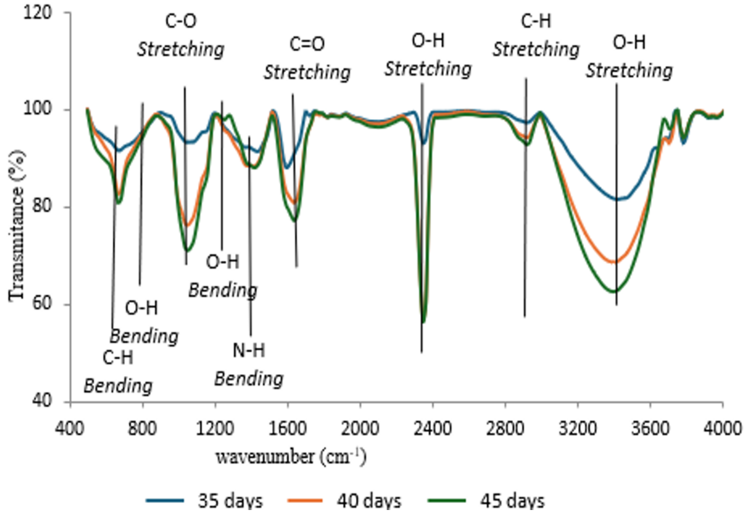

Fig. 2. The FTIR spectrum of the treated mycelium with a sleeves at opening times of 35, 40, and 45 days

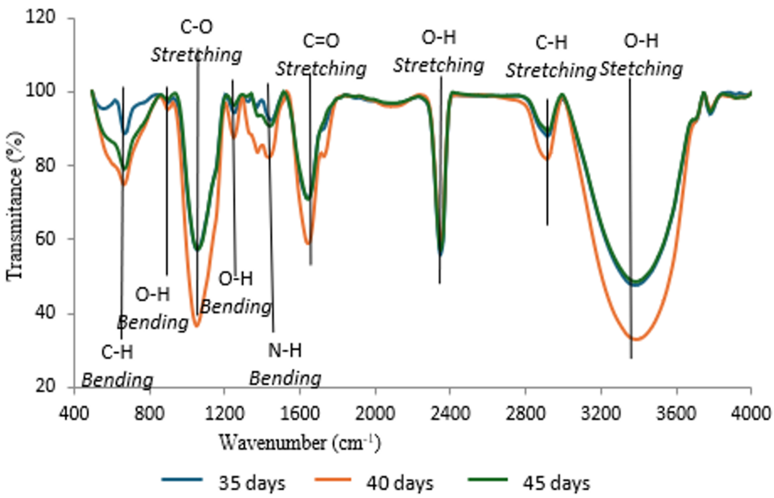

Fig. 3. The FTIR spectrum of the treated mushroom with a sleeves at opening times of 35, 40, and 45 days

The strong mycelium will develop into the fruiting body of the mushroom. The difference in treatment between using a sleeve and without a sleeve is evident in the transmittance values. High transmittance values are shown by the sleeve-treated mushrooms at 35 days, 40 days, and 45 days.

3.3 Optical properties of oyster mushroom sample

The characterization results of the mycelium and white oyster mushroom using FTIR yielded transmittance values. These values can be used to analyze the optical properties of the samples using the Kramers-Kronig method [9]. Based on this equation, the refractive index values will be obtained, consisting of the real refractive index (n) and the imaginary refractive index (k). The intersection of the n and k graphs at low wave numbers is referred to as Transversal Optic (TO), while the intersection of the n and k graphs at higher wave numbers is referred to as Longitudinal Optic (LO). Table 3 shows the LO and TO graph for the mycelium and mushroom when using the sleeve.

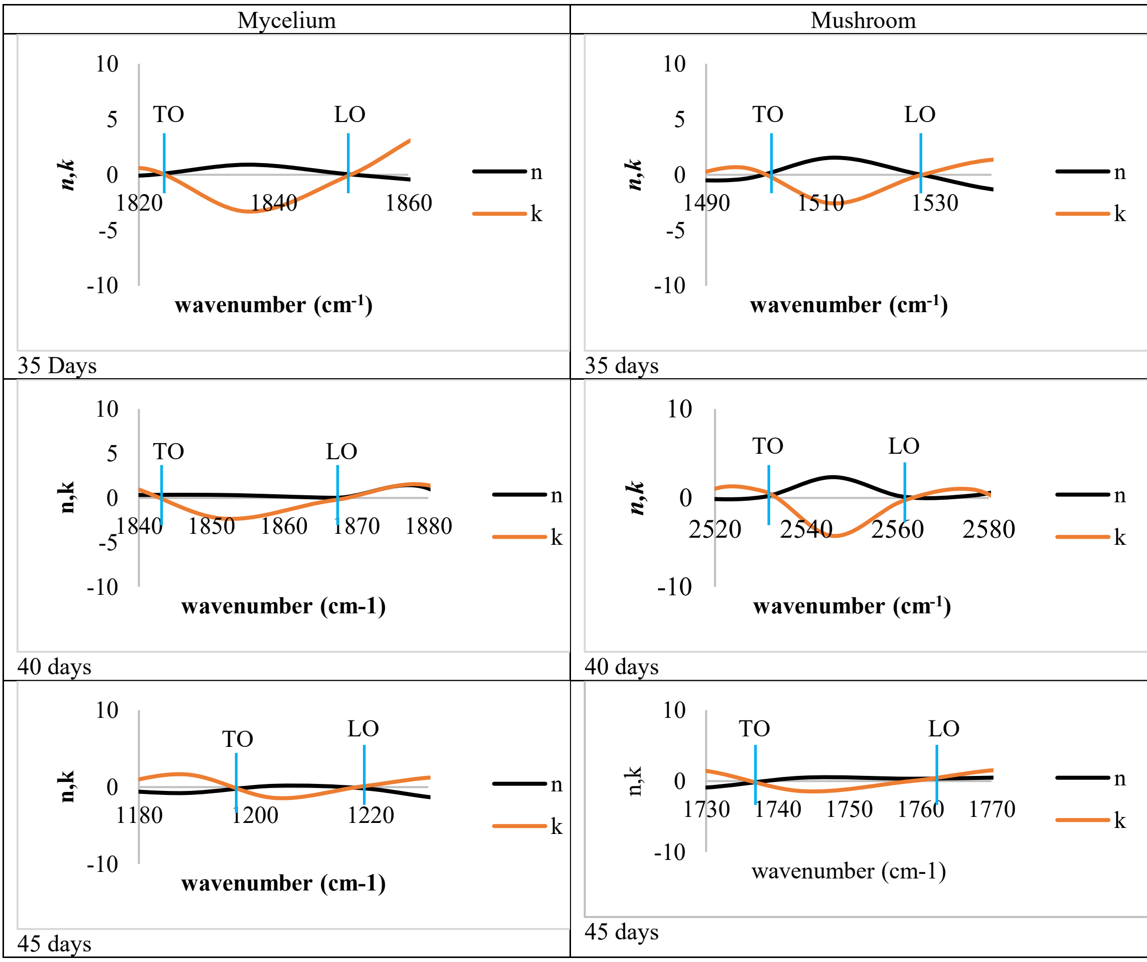

Fig. 4. LO and TO graph for the mycelium and mushroom when using the sleeve

Based on the image, it shows the values of LO and TO for mycelium and mushrooms. The difference in the opening time of the *baglog* indicates that the values of LO and TO at the 35-day opening time have values at smaller wave numbers, while at the 40-day opening time, the values of LO and TO shift towards larger wave numbers. Furthermore, at the 45-day opening time, the values of LO and TO are at shorter wave numbers than at the 40-day opening time. This indicates that the energy required for the optimal *baglog* opening time is greater than for the others. This is consistent with the relationship that photon energy is directly proportional to the wave number [10].

$$E = hc/\lambda$$

4 Conclusion

The characterization results of the mycelium and oyster mushrooms using FTIR at ages 35, 40, and 45 days indicate the presence of functional groups of the molecules C=O, C-H, C-O, and O-H, assuming a stretching process. The O-H molecule indicates the presence of water content in the mycelium and oyster mushrooms. The C=O, C-H, and C-O molecules indicate the presence of carbohydrate content in the mycelium and oyster mushrooms. The most optimal time to open the *baglog* is at 40 days, as indicated by the frequency of harvesting and the total mass of oyster mushroom harvest being relatively higher compared to the 35 and 45 days opening. This is suspected based on the results of the phonon vibration spectrum FTIR, where the 40-day age shows significant LO and TO values in the mycelium and oyster mushrooms, thus obtaining greater energy for the formation of the oyster mushroom harvest mass compared to the 35 and 45 days opening.

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