



Environmental Sustainable Utilization of Agricultural Waste-Based Sterilization for White Oyster Mushroom Cultivation: An FTIR Study of Optical Vibrational Properties

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Abstract

This study investigates the environmentally sustainable use of agricultural waste-based sterilization in white oyster mushroom (*Pleurotus ostreatus*) cultivation and its influence on mycelial optical vibrational properties. A small-scale industrial rice husk furnace was employed as an eco-friendly and low-cost sterilization system, providing an alternative to conventional fossil fuel-based energy sources. Potato Dextrose Agar (PDA) was used as the growth medium, while different sterilization levels were applied to obtain uncontaminated media and high-quality mycelial growth. Three sterilization levels were systematically analyzed using Fourier Transform Infrared (FTIR) spectroscopy to evaluate optical behavior at various cultivation stages. The optical properties of the mycelium were assessed through Longitudinal Optical (LO) and Transverse Optical (TO) vibrational modes derived from FTIR spectra. The results indicate that increasing the sterilization level leads to a consistent shift of both LO and TO modes toward higher wavenumbers, reflecting changes in molecular bonding and structural characteristics of the mycelium. Furthermore, longer boiling durations at 102 °C using the rice husk furnace resulted in higher transmittance values, attributed to the partial evaporation or reduction of certain organic compounds within the mycelium. Comparative analysis among cultivation stages shows that the planting spawn (F2) exhibits lower transmittance than the spreading spawn (F1), while the pure culture (F0) demonstrates the highest transmittance. These differences are closely related to variations in mycelial density, with F2 having the greatest mass per unit volume. Overall, the findings confirm that agricultural waste-based sterilization supports environmentally sustainable mushroom cultivation while significantly affecting the optical and structural properties of mycelium.

Keywords: fourier transform infrared, longitudinal optical, mycelium, rice husk furnace, transverse optical

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INTRODUCTION

White oyster mushroom (*Pleurotus ostreatus*) is widely recognized as a nutritionally valuable food source and an important component of sustainable agriculture systems. It is considered an alternative protein-rich food due to its balanced nutritional composition and relatively low production cost compared to animal-based protein sources. According to [Irzaman et al., \(2023\)](#), 100 g of fresh white oyster mushroom tissue contains approximately 17.12 g of protein, 2.60 g of fat, 38.87 g of carbohydrates, and provides 243.66 kcal of energy. In addition, white oyster mushrooms contain a high level of dietary fiber, reported to be around 30.25 g, along with an ash content of approximately 4.8 g. These nutritional characteristics make *P. ostreatus* an attractive functional food with increasing demand in both local and international markets ([Nayanathara Thathsarani Pilapitiya & Ratnayake, 2024](#)).

The growing demand for white oyster mushrooms has led to the expansion of cultivation practices among small- and medium-scale farmers. However, despite its economic potential, many mushroom growers continue to rely on spawn supplied by research institutions or commercial producers. This dependency often results in high production costs, which can be burdensome for small-scale farmers and limits the broader adoption of mushroom cultivation as a sustainable livelihood. Therefore, improving local spawn production methods (**Figure 1**) that are both cost-effective and environmentally friendly is essential to support sustainable mushroom farming ([Bhat et al., 2018](#); [Priyadarshi & Rhim, 2020](#); [Ulpathakumbura et al., 2026](#)).

Spawn production of *Pleurotus ostreatus* is commonly carried out using tissue culture techniques. This method involves isolating tissue from the fruiting body to obtain a pure culture, which serves as the initial stage for spawn multiplication. Tissue culture is favored because it enables the production of high-quality spawn with uniform growth characteristics. However, one of the major challenges associated with this technique is its high susceptibility to contamination by bacteria, molds, and yeasts. Even minimal contamination during the early stages of culture can severely affect mycelial growth and compromise spawn quality, ultimately reducing yield and economic viability ([Fadhallah et al., 2022](#)).

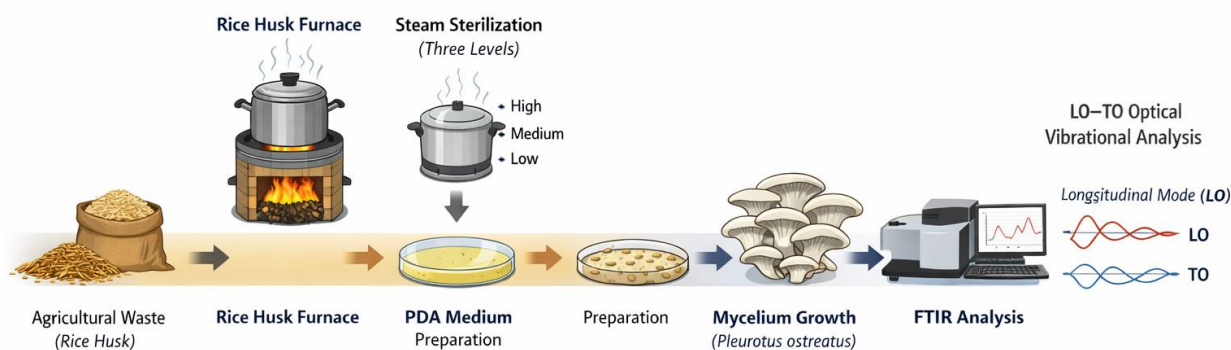


Figure 1. Sustainable oyster mushroom spawn production process

To minimize contamination, proper sterilization of the growth medium is a critical step in spawn production. Sterilization is necessary because prepared culture media often retain microbial contaminants that can outcompete mushroom mycelium for nutrients. Effective sterilization ensures the inactivation of various microorganisms, including bacteria, fungi, and yeasts, thereby creating favorable conditions for mycelial development. Nevertheless, achieving consistent sterilization requires careful control of temperature and duration, which can be challenging for farmers using traditional or low-cost equipment ([Kusumawati et al., 2025](#)).

In practical settings, the sterilization process demands considerable attention, labor, and energy input. As a result, many farmers prefer to purchase ready-to-use white oyster mushroom spawn at relatively high market prices rather than producing their own. This situation is particularly unfavorable for small-scale producers, who often operate with limited resources. Consequently, there is a need for alternative sterilization approaches that are not only effective but also affordable, simple to implement, and environmentally sustainable ([Kasayanond et al., 2019](#); [Meijaard et al., 2019](#); [Stanley et al., 2025](#)).

Various substrates have been used in mushroom spawn production, including rice husk, corn, and potato-based media. Among these, rice husk is an abundant agricultural by-product in many developing countries and is often underutilized or disposed of as waste. Recent studies have highlighted the potential of rice husk as a sustainable energy source when used as fuel in small-scale furnaces. Rice husk furnaces offer an environmentally friendly alternative to fossil fuel-based heating systems by converting agricultural waste into usable thermal energy (Abdurrahman et al., 2019).

In contrast, potato-based media, commonly referred to as Potato Dextrose Agar (PDA), are widely used in laboratory-scale mushroom cultivation due to their high carbohydrate content and suitability for mycelial growth. PDA is typically prepared by boiling potatoes for approximately 10 minutes during medium formulation. While PDA provides an excellent nutrient source, its preparation and sterilization still require reliable heating methods to ensure the elimination of microbial contaminants (Riana et al., 2025).

Integrating agricultural waste-based energy systems, such as rice husk furnaces, into mushroom cultivation practices offers a promising pathway toward environmental sustainability. By utilizing rice husk as a fuel source for sterilization, farmers can reduce reliance on fossil fuels, lower production costs, and contribute to waste valorization. However, beyond ensuring sterility, the sterilization process may also influence the physical and chemical properties of the resulting mycelium, which remain insufficiently explored (Putranto et al., 2025).

One effective approach to investigating these changes is through Fourier Transform Infrared (FTIR) spectroscopy. FTIR is a powerful analytical technique widely used to characterize molecular bonding, functional groups, and vibrational properties of biological materials. In mushroom research, FTIR has been applied to study biochemical composition, structural changes, and interactions between mycelium and growth substrates. Analysis of optical vibrational properties, particularly Longitudinal Optical (LO) and Transverse Optical (TO) modes, can provide valuable insights into structural modifications induced by different cultivation and sterilization conditions. Despite the growing interest in sustainable mushroom cultivation, limited studies have addressed the relationship between agricultural waste-based sterilization methods and the optical vibrational properties of mushroom mycelium. Understanding this relationship is important not only for optimizing spawn quality but also for assessing the broader implications of eco-friendly cultivation systems on mycelial structure and performance (Appu et al., 2021; Santos et al., 2024).

Based on these considerations, this study aims to develop high-quality white oyster mushroom spawn while minimizing contamination levels through the use of environmentally sustainable sterilization methods. The spawn was cultivated using Potato Dextrose Agar (PDA) medium, prepared by boiling potatoes for 10 minutes. Sterilization was performed using a steam vessel (locally known as a dandang) heated by a small-scale industrial rice husk furnace. Three different sterilization levels were applied to evaluate their effectiveness in suppressing contamination and influencing mycelial properties. Subsequently, the cultivated samples were characterized using FTIR spectroscopy to analyze their optical vibrational properties, with particular focus on Longitudinal Optical (LO) and Transverse Optical (TO) modes (Antarnusa, 2022; Farahmandjou et al., 2016).

The findings of this study are expected to contribute to the development of low-cost, environmentally sustainable mushroom cultivation practices while providing new insights into the relationship between sterilization conditions and mycelial optical characteristics. Such knowledge may support the broader adoption of agricultural waste-based technologies in sustainable food production systems.

METHOD

Sample Preparation and Spawn Cultivation

Sample preparation in this study involved the production of white oyster mushroom (*Pleurotus ostreatus*) spawn followed by optical characterization using Fourier Transform Infrared (FTIR) spectroscopy, as reported in previous studies. The spawn production process began with the preparation of Potato Dextrose Agar (PDA) medium, which consisted of potatoes, agar, and dextrose as the primary components. This medium served as the initial growth substrate for mycelial development (Har et al., 2021).

The first stage of cultivation produced the pure culture sample, denoted as F0. Subsequently, the F0 culture was isolated and propagated to obtain the spreading spawn (F1), which was further developed into the planting spawn (F2). These three samples (F0, F1, and F2) represent successive stages of spawn production as well as different sterilization levels applied during PDA preparation.

Sterilization Procedure Using Rice Husk Furnace

Sterilization of the PDA medium was conducted using a steam vessel (dandang) heated by a small-scale industrial rice husk furnace, utilizing agricultural waste as an environmentally sustainable energy source. Three levels of sterilization were applied to evaluate their effectiveness in minimizing contamination and influencing mycelial properties. Sterilization Level 1 involved steaming the PDA medium for a total of 100 minutes. This process consisted of approximately 20 minutes to raise the water temperature to boiling (100 °C), followed by 80 minutes of continuous steaming. After sterilization, the medium was poured into test tubes under aseptic conditions and stored in a sterile box for 24 hours prior to inoculation. Sterilization Level 2 followed the same procedure as Level 1. After 24 hours of storage in the sterile box, the PDA medium was subjected to a second steaming cycle of 100 minutes before use. Sterilization Level 3 was carried out by repeating the process once more. After completion of Level 2 sterilization and 24 hours of sterile storage, the medium underwent an additional 100-minute steaming cycle. This stepwise sterilization approach was designed to compare contamination suppression and mycelial quality under increasing sterilization intensity.

FTIR Characterization and Optical Analysis

The three sterilized samples (F0, F1, and F2) were characterized using FTIR spectroscopy to investigate differences in optical vibrational properties at each stage of spawn production. FTIR spectra were recorded in terms of wavenumber and percent transmittance. The optical properties were analyzed based on the Longitudinal Optical (LO) and Transverse Optical (TO) vibrational modes. To obtain LO and TO values, the FTIR transmittance data were further processed using analytical calculations based on the Kramers–Kronig (KK) relations (Irzaman et al., 2023). From these calculations, the complex refractive index was derived, consisting of the real part (n) and the imaginary part (k), expressed as follows:

$$n(\omega) = \frac{1-R(\omega)}{1+R(\omega)-2\sqrt{R(\omega)}\cos\varphi(\omega)} \quad (1)$$

$$k(\omega) = \frac{2\sqrt{R(\omega)}\sin\varphi(\omega)}{1+R(\omega)-2\sqrt{R(\omega)}\cos\varphi(\omega)} \quad (2)$$

where $R(\omega)$ represents the reflectance, ω denotes the wavenumber, and $\varphi(\omega)$ is the phase shift. The phase shift was calculated using the Kramers–Kronig relation as follows (Irzaman et al., 2023):

$$\varphi(\omega) = -\frac{4\omega_j}{\pi} x\Delta\omega x \sum_i \frac{\ln(\sqrt{R(\omega)})}{\omega_i^2 - \omega_j^2} \quad (3)$$

In this expression, ω_i represents the wavenumber at odd-numbered series, while ω_j corresponds to the wavenumber at even-numbered series. The derived optical constants were subsequently used to identify LO and TO vibrational modes and to analyze their variation with sterilization level and spawn development stage.

RESULTS AND DISCUSSION

The success of white oyster mushroom (*Pleurotus ostreatus*) cultivation is strongly influenced by the quality of the spawn, which depends on the purity and nutrient availability of the growth medium. In this study, Potato Dextrose Agar (PDA) was used as the primary growth medium, and variations in sterilization level were applied to evaluate their effectiveness in minimizing contamination and supporting mycelial development. Proper sterilization is crucial because residual microorganisms or

wild fungi present in inadequately sterilized media can inhibit mycelial growth and lead to cultivation failure. The success rate of PDA preparation under different sterilization levels is summarized in **Table 1**. The symbol (✓) indicates successful mycelial growth, while (-) indicates the absence of growth.

Table 1. Success rate of PDA preparation

Replication	Sterilization Level		
	Level 1	Level 2	Level 3
1	✓	✓	✓
2	✓	✓	✓
3	-	-	✓
4	-	-	-

✓ : growth observed; (-) : no growth observed

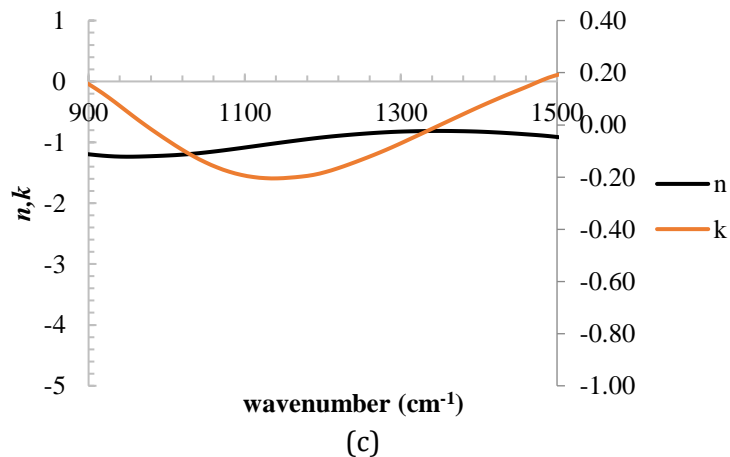
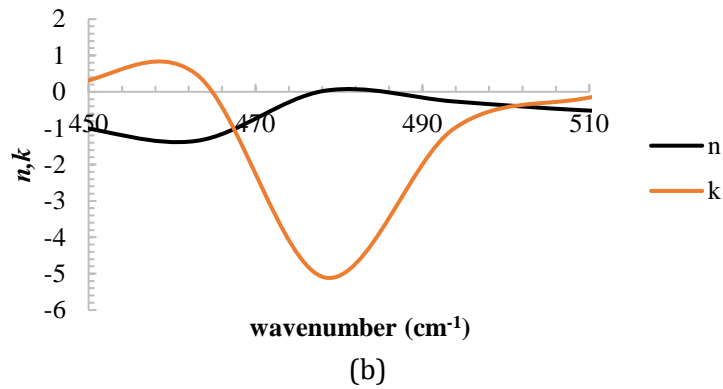
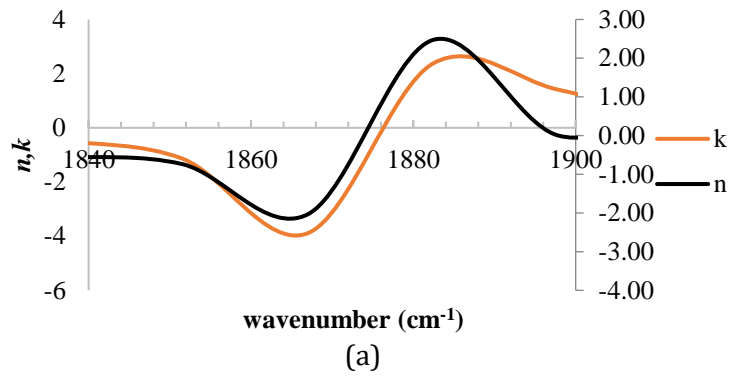


Figure 2. LO and TO values of sterilized samples: (a) F0 (Level 1); (b) F1 (Level 2); (c) F2 (Level 3)

The results demonstrate that Sterilization Level 3 produced the highest success rate of mycelial growth compared to Levels 1 and 2. This improvement is attributed to repeated steaming at maximum temperature, which more effectively eliminated bacterial and fungal contaminants in the PDA medium. In contrast, incomplete sterilization at lower levels likely allowed residual microorganisms to survive and compete with the mycelium for nutrients. After inoculation, the cultures were maintained at an incubation temperature of approximately 29 °C, which is considered optimal for white oyster mushroom mycelial growth and further supported successful development (Abdurrahman et al., 2019; Nayanathara Thathsarani Pilapitiya & Ratnayake, 2024). The optical properties of the cultivated samples were analyzed using FTIR spectroscopy, and the derived Longitudinal Optical (LO) and Transverse Optical (TO) modes are presented in Figure 1. In this analysis, the TO mode is identified by the intersection point between the real (n) and imaginary (k) parts of the refractive index at lower wavenumbers, while the LO mode corresponds to their intersection at higher wavenumbers.

Based on **Figure 2**, variations in sterilization level resulted in noticeable shifts in both LO and TO modes. The TO values for samples F0, F1, and F2 were observed at wavenumbers of 1851 cm^{-1} , 470 cm^{-1} , and 1033 cm^{-1} , respectively, while the corresponding LO values appeared at 1897 cm^{-1} , 490 cm^{-1} , and 1326 cm^{-1} . The samples subjected to Sterilization Levels 1 and 3 exhibited LO and TO modes at higher wavenumbers compared to Level 2, indicating changes in molecular bonding and structural organization within the mycelium. These shifts suggest that increased sterilization intensity influences the biochemical composition and optical vibrational behavior of the mycelium. Since photon energy is directly proportional to wavenumber, higher LO and TO values imply higher energy vibrational modes, which may be associated with reduced organic impurities and increased structural uniformity in the mycelium. Overall, the results confirm that agricultural waste-based sterilization not only enhances spawn quality by reducing contamination but also significantly affects the optical properties of white oyster mushroom mycelium.

In relation to the study objective and the proposed concept of environmentally sustainable utilization of agricultural waste-based sterilization, the present findings demonstrate a novel integration of eco-friendly energy systems with optical characterization of biological materials. Unlike conventional studies that primarily focus on contamination reduction or yield improvement, this work reveals that sterilization driven by a rice husk furnace not only enhances spawn success rates but also systematically modifies the optical vibrational properties of white oyster mushroom mycelium, as evidenced by the observed shifts in LO and TO modes. The novelty of this study lies in establishing a direct link between agricultural waste-based sterilization intensity and FTIR-derived optical responses of mycelium, providing a physicochemical perspective on sustainable mushroom cultivation. By correlating sterilization level, mycelial development stage, and optical behavior, this research extends the understanding of how environmentally sustainable practices can influence both biological performance and material properties of mycelium. These insights highlight the potential of rice husk-based sterilization systems as not only a low-cost and environmentally responsible alternative for spawn production but also a controllable parameter for tailoring mycelial structural characteristics, thereby supporting the broader adoption of sustainable and resource-efficient cultivation technologies.

Effect of Agricultural Waste-Based Sterilization on Spawn Success

The effectiveness of rice husk-based steam sterilization was evaluated through the success rate of mycelial growth on PDA medium under three sterilization levels. The results (Table 1) show that Sterilization Level 3 achieved the highest success rate (75%), whereas Levels 1 and 2 both resulted in 50% growth success. The improved performance observed at Level 3 is attributed to repeated steaming at maximum temperature, which more effectively eliminated bacterial and fungal contaminants. Insufficient sterilization at lower levels likely allowed residual microorganisms to survive, thereby competing with the mycelium for nutrients and reducing growth success.

From a sustainability perspective, increasing sterilization effectiveness directly contributes to improved resource efficiency. Higher spawn success rates reduce material losses, repeated media preparation, and additional energy consumption associated with failed cultures. Consequently, optimizing sterilization intensity not only enhances biological performance but also minimizes waste generation within the production cycle. Moreover, the use of rice husk as a fuel source represents a

form of agricultural waste valorization. Instead of relying on fossil-fuel-based heating systems, the sterilization process utilized locally available biomass residues, supporting circular economy principles and reducing dependency on external energy sources.

FTIR Spectral Characteristics of Mycelium under Different Sterilization Levels

FTIR spectroscopy was employed to examine potential physicochemical changes in the cultivated mycelium induced by variations in sterilization intensity. The spectra revealed characteristic absorption bands commonly associated with fungal biomaterials, including regions corresponding to O–H stretching, C–H vibrations, amide bonds, and carbohydrate-related functional groups. Differences in spectral features among samples F0 (Level 1), F1 (Level 2), and F2 (Level 3) indicate that sterilization intensity influenced molecular organization within the mycelial structure. Variations in peak positions and optical parameters suggest changes in bonding environments and structural ordering, potentially associated with improved substrate purity and reduced microbial interference. These findings suggest that sterilization does not merely function as a contamination control step but may also affect the biochemical and structural characteristics of the resulting mycelium.

LO–TO Optical Vibrational Analysis

The derived Transverse Optical (TO) and Longitudinal Optical (LO) modes were identified from the intersection of the real (n) and imaginary (k) components of the refractive index. Distinct shifts in both TO and LO modes were observed across sterilization levels. The TO values for F0, F1, and F2 were recorded at 1851 cm^{-1} , 470 cm^{-1} , and 1033 cm^{-1} , respectively, while the corresponding LO values appeared at 1897 cm^{-1} , 490 cm^{-1} , and 1326 cm^{-1} . These variations demonstrate that sterilization intensity influenced the vibrational behavior of molecular bonds within the mycelium.

Since vibrational energy is proportional to wavenumber, higher LO and TO positions correspond to higher vibrational energy states. The relatively higher values observed in Level 3 suggest a more structurally uniform or less impurity-influenced mycelial network. This may be explained by improved nutrient availability and reduced microbial competition during growth, leading to more consistent biochemical development. The separation between LO and TO modes further reflects changes in dipole–dipole interactions and molecular ordering. Such structural modifications highlight the sensitivity of optical vibrational properties to cultivation conditions. Importantly, these results demonstrate that agricultural waste-based sterilization does not negatively impact material integrity; rather, it systematically influences physicochemical properties of the mycelium.

Sustainability Implications of Rice Husk–Based Sterilization

Beyond contamination control, the integration of a rice husk furnace into spawn production represents a practical application of biomass-based energy systems in small-scale agriculture. This approach contributes to sustainability through:

1. Waste valorization – converting rice husk residues into useful thermal energy.
2. Reduced fossil fuel reliance – minimizing dependence on LPG or electricity-based sterilization.
3. Improved production efficiency – higher spawn success reduces repeated processing and resource consumption.
4. Local adaptability – the technology can be implemented in rural settings with minimal infrastructure.

Unlike conventional studies that focus solely on yield or contamination metrics, this work establishes a link between environmentally sustainable sterilization technology and physicochemical characteristics of fungal biomaterials. The correlation between sterilization intensity and LO–TO optical response provides a novel perspective on how sustainable processing conditions influence both biological performance and material structure. These findings support the broader adoption of agricultural waste-based energy systems in mushroom cultivation as a low-cost, environmentally responsible, and scientifically validated alternative to conventional sterilization methods.

CONCLUSION

Based on the results of this study, it can be concluded that increasing the sterilization level using an agricultural waste-based rice husk furnace significantly influences the optical vibrational properties of white oyster mushroom (*Pleurotus ostreatus*) mycelium. Higher sterilization intensity resulted in systematic shifts of both Longitudinal Optical (LO) and Transverse Optical (TO) modes toward higher wavenumbers, indicating changes in molecular bonding and structural organization of the mycelium. In addition, longer boiling durations at 102 °C were associated with increased transmittance values, which are attributed to the reduction or partial evaporation of certain organic compounds during the sterilization process. Comparative analysis among cultivation stages showed that the planting spawn (F2) exhibited lower transmittance than the spreading spawn (F1), while the pure culture (F0) displayed the highest transmittance. These differences are closely related to variations in mycelial density, where F2 has the greatest mass per unit volume, followed by F1 and F0. The novelty of this study lies in demonstrating that an environmentally sustainable sterilization system based on agricultural waste not only improves spawn quality by reducing contamination but also directly affects the optical vibrational behavior of mycelium, as revealed through FTIR-derived LO and TO modes. By establishing a clear relationship between sterilization level, spawn development stage, and optical properties, this work provides new insights into how eco-friendly cultivation practices can simultaneously enhance biological performance and influence the physicochemical characteristics of mushroom mycelium. These findings highlight the potential of rice husk-based sterilization as a low-cost, sustainable, and controllable approach for optimizing white oyster mushroom spawn production.

AUTHOR CONTRIBUTIONS

Conceptualization, I; methodology, I, EA, and NPH; software, I and RS; validation, I, EA, and MMR; formal analysis, I and NPH; investigation, HS and RPJ; resources, MI and RS; data curation, EA and NPH; writing—original draft preparation, EA and HS; writing—review and editing, I, MMR, and MI; visualization, RS and NPH; supervision, I; project administration, MI; funding acquisition, I.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest concerning the publication of this article. The authors also confirm that the data and the article are free of plagiarism.

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