



The Effect Of Coconut Water (*Cocos nucifera*) Concentration In Ramie Wood Chip (*Boehmeria nivea*) Substrate On The Growth Of Brown Oyster Mushroom (*Pleurotus cystidiosus*)

Qurrotu Ainun Nisa^{1*}, Ruly Budiono², Suryana³, Indri Wulandari⁴, M. Agung Triyudha Agustiana⁵

^{1,2}Universitas Padjadjaran, Biologi Fakultas MIPA, Kabupaten Sumedang, Jawa Barat

^{3,4,5}Universitas Padjadjaran, Biologi Fakultas MIPA, Kabupaten Sumedang, Jawa Barat

*Corresponding author email: qurrotu19002@mail.unpad.ac.id

Abstract

The cultivation of brown oyster mushrooms (*Pleurotus cystidiosus*) often faces constraints due to the limited availability of suitable growth media. This study aims to evaluate the potential of ramie wood chips as an alternative substrate and coconut water as a nutritional supplement to enhance mushroom growth. Coconut water is rich in plant growth regulators (PGRs) such as cytokinins, while ramie chips contain high cellulose and lignin content, making them a promising combination for a cultivation medium. The research was conducted using a Completely Randomized Design (CRD) consisting of four coconut water concentrations: 0% (K0), 25% (K1), 50% (K2), and 100% (K3), with six replications. The observed parameters included mycelial growth rate, time of first harvest (Days After Inoculation/DAI), harvest frequency, number of fruiting bodies, pileus diameter, and substrate weight loss. The data obtained were analyzed using Analysis of Variance (ANOVA). Significant results were further evaluated using Duncan's Multiple Range Test (DMRT) at a 5% significance level to determine the differences between treatments.

Keywords: Brown oyster mushroom, Coconut water, Mushroom cultivation, Ramie chips, Substrate weight loss.

1. Introduction

Brown oyster mushroom (*Pleurotus cystidiosus*) is a functional food commodity from the Basidiomycota group that possesses high economic value due to its delicious flavor and longer shelf life compared to white oyster mushrooms. Besides being a source of protein and fiber, this mushroom contains various bioactive compounds that serve as antioxidants and cholesterol-lowering agents (Rosmiah et al., 2020; Sumarmi, 2006). However, oyster mushroom productivity in Indonesia has been reported to fluctuate, partly due to the limited availability of conventional planting media.

Traditionally, oyster mushroom cultivation has heavily relied on wood sawdust as the primary source of lignocellulose. With the increasing price and limited supply of sawdust, the search for alternative media from agricultural waste has become crucial. Such waste must meet criteria such as abundant availability, affordability, and suitable nutrient content to support the metabolism of wood-decaying fungi.

One material with great potential is ramie wood chips (*Boehmeria nivea*), a byproduct of the ramie fiber decortication process. Accounting for 96–97% of the wet stem weight, ramie chips contain high cellulose levels, ranging from 44.82% to 91% (Aprilia Lestari & Bagus Priambodo, 2020; Vigneshwaran et al., 2020). This cellulose content serves as the primary energy source for the *Pleurotus* genus during the decomposition process to form fruiting bodies; however, its utilization in Indonesia remains minimal.

Despite being rich in carbon, pure lignocellulosic-based media like ramie chips often require additional nutrient supplementation to accelerate the vegetative phase. Coconut water (*Cocos nucifera*) can be used as an organic supplement because it contains a complete range of minerals (particularly Potassium), vitamins, and simple sugars. Its primary advantage lies in the presence of natural Plant Growth Regulators (PGRs) such as cytokinins, auxins, and gibberellins, which play a significant role in stimulating hyphal cell division and mycelial differentiation (Ananto, 2021; Mudaningrat & Nada, 2021).

To date, research on the optimization of brown oyster mushroom growth on ramie wood chip media with the addition of coconut water remains very limited. Variations in coconut water concentration are suspected to produce different growth responses due to the influence of the growth hormones contained therein. Therefore, a study on the

precise dosage is necessary to achieve maximum production efficiency without the risk of contamination caused by excessive nutrients.

This study aims to analyze the effect of varying coconut water concentrations on the mycelial growth rate and harvest yield of brown oyster mushrooms on ramie wood chip-based media, as well as to determine the most optimal concentration level. The results of this research are expected to provide new scientific references in the field of applied mycology and offer practical solutions for farmers to utilize ramie fiber industry waste into high-value food products.

2. Literature Review

Pleurotus cystidiosus, commonly known as the brown oyster mushroom, is a high-value functional food commodity distinguished by its superior flavor and significantly longer shelf life compared to the white oyster mushroom (*P. ostreatus*). Nutritional analyses indicate that this species contains high essential protein levels, ranging from 10.5% to 30.4%, along with vital bioactive compounds such as lovastatin and β -D-glucan, which function as cholesterol-lowering and anti-tumor agents (Sumarmi, 2006; Hasanah et al., 2023). Genetically, *P. cystidiosus* exhibits a slower vegetative phase compared to other *Pleurotus* species, requiring a stable and nutrient-rich environment for optimal primordia formation (Budiono et al., 2024; Woo et al., 2025). As a lignocellulolytic saprophytic organism, the cultivation success of this mushroom depends heavily on the quality of the substrate provided.

Ramie wood chips (*Boehmeria nivea*), a byproduct of the fiber decortication process, have emerged as a potent alternative to conventional sawdust due to their abundant availability and high nutrient potential. These chips account for nearly 96–97% of the total wet stem weight of the ramie plant and contain a high cellulose content of up to 91%, alongside a lignin content of approximately 18.26% (Lestari & Priambodo, 2020; Vigneshwaran et al., 2020). However, the complex structure of lignin necessitates specialized enzymatic decomposition. Fungi of the *Pleurotus* genus utilize extracellular enzymes, specifically laccase and manganese peroxidase, to degrade these complex polymers into simpler energy sources (Hafizah & Aspiani, 2016; Widiwurjani et al., 2021).

To further optimize growth and accelerate the incubation period, organic supplementation using coconut water (*Cocos nucifera*) is often implemented. Coconut water serves as a versatile supplement rich in Potassium (K), vitamins, and simple sugars. Its primary advantage in mycology lies in the presence of natural Plant Growth Regulators (PGRs), including cytokinins, auxins, and gibberellins, which play a crucial role in stimulating hyphal cell division and mycelial differentiation (Ananto, 2021; Nugroho et al., 2023). The physiological response of *P. cystidiosus* to such supplementation follows specific biological mechanisms. Low doses can trigger Hormesis, a phenomenon where the mycelium gains increased post-harvest vitality and faster regeneration (Grich et al., 2025).

Conversely, excessive nutrient concentrations can lead to negative metabolic impacts. High concentrations create a hypertonic environment, resulting in osmotic stress where fluid is drawn out of the hyphal cells (exosmosis), forcing the fungus into a survival mode that halts longitudinal growth (Xu & Zwiazek, 2020; Branco et al., 2022). Furthermore, an overabundance of easily accessible sugars can trigger Carbon Catabolite Repression (CCR). In this state, the fungus prioritizes the metabolism of simple glucose and suppresses the production of cellulase enzymes, thereby reducing the efficiency of degrading the more complex lignocellulosic structures of the ramie substrate (Woo et al., 2025; Nurhayati et al., 2025). Environmental factors, such as relative humidity (RH) below the optimal range of 80-90%, may further restrict cellular expansion during the development of the mushroom's pileus (Money, 2025; Mustaqim, 2018).

3. Materials and Methods

3.1. Materials

Materials The primary material used in this study was ramie wood chips (*Boehmeria nivea*) as the main substrate, obtained from decortication waste. Supplementary materials for the media consisted of rice bran, corn bran, lime (CaCO_3), and gypsum (CaSO_4). The supplemental nutrient tested was young green coconut water diluted with water adjusted to pH 8. The fungal seeds used were F2 generation seeds of the brown oyster mushroom (*Pleurotus cystidiosus*).

3.2. Methods

3.2.1. Experimental Design

This study utilized an experimental method with a single-factor Completely Randomized Design (CRD). The treatments consisted of four concentration levels of coconut water: K0 (0%), K1 (25%), K2 (50%), and K3 (100%). Each treatment was replicated six times, resulting in a total of 24 experimental units.

3.2.2. Work Procedure

Substrate preparation (baglogs) was conducted by mixing ramie chips (80%), rice bran (15%), corn bran (3%), lime (1.4%), and gypsum (0.6%) with a moisture content of 60–70%. The baglogs were sterilized for 7 hours at temperatures ranging from 90–110°C. Inoculation of F2 seeds was performed aseptically, followed by an incubation phase at 28–30°C.

Coconut water application was carried out after the mycelium had adapted for 30 days of incubation. A total of 100 ml of coconut water solution, corresponding to the treatment concentration, was injected into the baglog at 10 different points. Maintenance was conducted in a mushroom house (*kumbung*) with routine daily watering to maintain humidity.

3.2.3. Observation Parameters and Data Analysis

All Observed parameters included the vegetative phase (mycelial growth rate) and the generative phase (days to first harvest/HSI, harvest frequency, number of fruiting bodies, pileus diameter, and substrate weight loss). The collected data were analyzed using one-way Analysis of Variance (ANOVA) at a 95% confidence level. If significant effects were found, a follow-up Duncan's Multiple Range Test (DMRT) was performed at $\alpha = 0.05$.

4. Results and Discussion

4.1. Mycelial Growth Rate (cm/day)

Mycelium is the vegetative network of fungi consisting of fine branching filaments called hyphae. Calculating the mycelial growth rate is a crucial indicator for measuring the fungus's ability to absorb nutrients from the growth substrate. The Analysis of Variance (ANOVA) indicated that the application of coconut water significantly influenced the mycelial growth rate ($F_{\text{calculated}} 37.125 > F_{\text{table}} 3.10$).

Table 1: Average Mycelial Growth Rate (30 DAI – 53 DAI)

Treatment (K)	Coconut Water Concentration	Mycelial Growth Rate (cm/day)
K0 (Control)	0%	0.34 ^a
K1	25%	0.29 ^a
K2	50%	0.32 ^a
K3	100%	0.08 ^b

Note:

Values followed by different letters indicate a significant difference according to the 5% DMRT test.

The DMRT follow-up test results showed that treatment K3 (100% coconut water) produced the significantly slowest growth rate (0.08 cm/day). The stagnation phenomenon observed in K3 after day 31 is suspected to be a result of **osmotic stress**. An excessively high nutrient concentration creates a hypertonic environment, causing fluid within the hyphal cells to be drawn out (**exosmosis**) (Xu & Zwiazek, 2020; Branco et al., 2022). Consequently, the mycelium shifts into a survival mode to restore cellular equilibrium rather than continuing longitudinal growth (Costa-Mattioli & Walter, 2020; Branco et al., 2022). Meanwhile, the fastest rate in K0 (0.34 cm/day) suggests that without additional coconut water, the mycelium grows aggressively to locate nutrient sources.

The initial development of mycelial colonization on the ramie substrate for each treatment was documented at 30 DAI (Days After Inoculation), as shown in **Figure 4.1**. At 30 DAI, mycelial growth across all experimental units (K0, K1, K2, and K3) remained relatively uniform and did not show contrasting differences. This occurred because the coconut water treatments had not yet been applied during the 0–30 DAI period; nutrient injection was only performed on day 31.

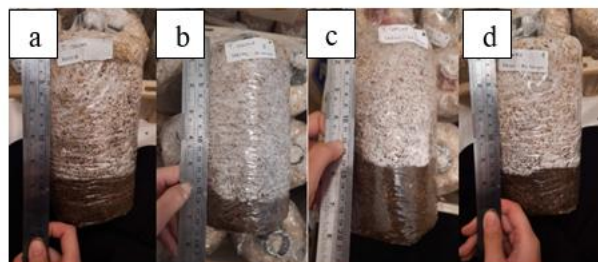


Figure 1: Mycelial growth of *P. cystidiosus* at 30 DAI: (a) K0; (b) K1; (c) K2; (d) K3.

Significant visual differences only began to be observed at the end of the vegetative phase (53 DAI), as presented in **Figure 4.2**. Treatments K0, K1, and K2 reached a "fully colonized" state, where white mycelium evenly covered the entire substrate. In contrast, treatment K3 showed clear stagnation in mycelial growth.



Figure 2: Mycelial growth of *P. cystidiosus* at 53 DAI: (a) K0; (b) K1; (c) K2; (d) K3

4.2. First Harvest Time (DAI)

The first harvest time was calculated from the moment of inoculation until the fruiting bodies were ready for harvest. Statistical results showed that the application of coconut water did not significantly affect this parameter ($F_{\text{calculated}} < F_{\text{table}}$).

Table 2: Average First Harvest Time

Treatment (K)	Average (DAI)
K0 (Control)	62.17 ^a
K1 (25%)	62.17 ^a
K2 (50%)	61.67 ^a
K3 (100%)	62.17 ^a

The uniform time range between 61–62 DAI proves that *P. cystidiosus* genetically possesses a slower vegetative phase compared to white oyster mushrooms (Budiono et al., 2024; Woo et al., 2025). Besides genetic factors, the lignin content in ramie wood chips reaching 18.26%, requires a longer and more stable enzymatic decomposition process (facilitated by laccase and manganese peroxidase enzymes) before the mycelium can accumulate into fruiting body primordia (Budiono et al., 2024; Hafizah & Aspiani, 2016; Widiwurjani et al., 2021).

4.3. Fruiting Body Productivity (Number of Fruiting Bodies and Harvest Frequency)

Productivity was observed through two indicators: the total number of fruiting bodies and the harvest frequency during the study period.

Table 3: Recapitulation of Brown Oyster Mushroom Productivity (53 DAI – 98 DAI)

Treatment (K)	Mean Number of Fruiting Bodies	Mean Harvest Frequency
K0 (Control)	22.50 ^a	2.67 ^a
K1 (25%)	14.50 ^a	3.00 ^a
K2 (50%)	17.17 ^a	2.00 ^a
K3 (100%)	10.00 ^a	1.83 ^a

Despite the high statistical variation (**Figure 4.3**), a trend emerged showing that K0 produced the highest number of mushrooms (22.50 units). This is suspected to be an emergency reproductive strategy (**stress-induced fruiting**). In media with low supplemental nutrients, the fungus receives a strong signal to immediately produce as many spores as possible for generational survival. On the other hand, K1 (25%) showed the most stable harvest frequency (3 times). This is an effect of **Hormesis**, where low doses of cytokinins and auxins from coconut water enhance post-harvest mycelial vitality, allowing for more frequent regeneration (Grich et al., 2025).

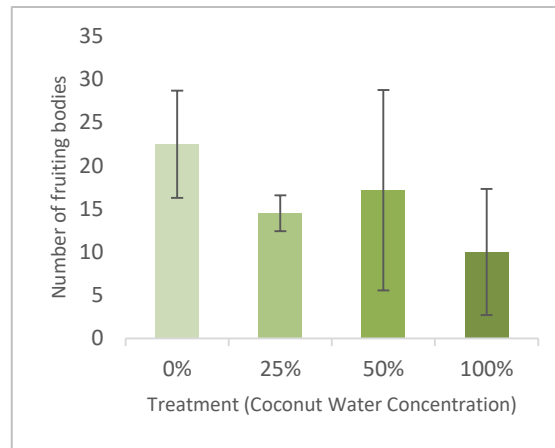


Figure 3: Average number of *P. cystidiosus* fruiting bodies at various coconut water concentrations. Vertical lines indicate standard deviation ($n = 6$).

4.4. Physical Characteristics and Pileus Morphology

Harvest quality was assessed based on pileus (cap) diameter and the physical appearance of the fruiting bodies. The average pileus diameter at the first harvest is presented in **Table 4.4**:

Table 4: Average Pileus Diameter of the First Harvest

Treatment (K)	Mean Diameter (cm)
K0 (Control)	5.06 ^a
K1 (25%)	6.37 ^a
K2 (50%)	5.96 ^a
K3 (100%)	5.33 ^a

Analysis showed no significant difference in pileus diameter between treatments. This was influenced by limiting environmental factors (Money, 2025; Mustaqim, 2018), particularly the relative humidity (RH) in the mushroom house, which averaged around 75%, below the optimal standard for oyster mushrooms (80-90%) (Budiono et al., 2024). This low humidity restricted maximum cellular expansion of the pileus across all experimental units. Although statistically similar, visual differences in morphology were observed as documented in **Figure 4.4**.

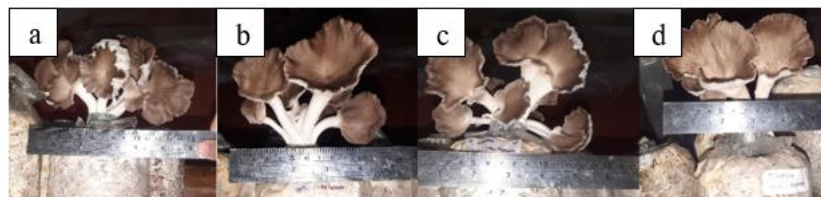


Figure 4: Morphology of *P. cystidiosus* Fruiting Bodies at First Harvest: (a) K0; (b) K1; (c) K2; (d) K3.

Based on **Figure 4.4**, differences in morphological quality were evident, particularly in pileus color and expansion. Treatment K1 (25% coconut water) provided the best morphological performance, with bright brown, thick-textured, and sturdy caps (b). Conversely, in K3 (100% coconut water), the fruiting bodies were smaller and paler (d). This resulted from the osmotic stress mechanism inhibiting cellular expansion and potential shifts in substrate pH due to undiluted coconut water, which is not ideal for fungal pigment synthesis (Limbad et al., 2023). This indicates that excessive coconut water doses actually decrease the aesthetic and economic value of brown oyster mushrooms.

4.5. Substrate Weight Loss

Substrate weight loss indicates the efficiency of ramie wood chip degradation by the fungus's enzymatic activity. ANOVA showed a highly significant effect ($F_{\text{calculated}} 64.392 > F_{\text{table}} 3,10$).

Table 5: Average Substrate Weight Loss

Treatment (K)	Average Weight Loss (grams)
K0 (Control)	397.50 ^b
K1 (25%)	302.17 ^{ab}
K2 (50%)	326.67 ^{ab}
K3 (100%)	142.00 ^a

K0 experienced the greatest weight loss (397.50 grams). This proves a nutrient stress response, where the mycelium works extra aggressively to decompose the ramie fibers to obtain energy due to the lack of external nutrient input (Nurhayati et al., 2025). In contrast, K3 showed the lowest weight loss (142.00 grams) due to the Carbon Catabolite Repression (CCR) phenomenon (Woo et al., 2025). The abundance of simple sugars from undiluted coconut water suppressed the fungus's cellulase enzyme production, causing the fungus to be "reluctant" to decompose the more complex structure of the ramie wood chips.



Figure 5: Condition of *P. cystidiosus* baglogs during the non-productive phase: (a) K0; (b) K1; (c) K2; (d) K3.

5. Conclusion

The addition of coconut water as a natural Plant Growth Regulator (PGR) on ramie wood chip substrate is proven to have a significant effect on the growth effectiveness of the brown oyster mushroom (*P. cystidiosus*). The 25% concentration (K1) was identified as the most optimal dose, yielding the highest harvest frequency (3 times) and the best pileus morphological quality (thick, sturdy, and bright). In contrast, the K3 treatment (100%) resulted in the significantly slowest mycelial growth rate of 0.08 cm/day (compared to 0.34 cm/day in the control) due to an osmotic stress mechanism that inhibits hyphal elongation. Furthermore, the lowest substrate weight loss was observed in K3 at 142.00 grams (compared to 397.50 grams in the control), indicating the phenomenon of Carbon Catabolite Repression (CCR), where an abundance of simple sugars suppresses cellulase enzyme production. Although there were no significant effects on the first harvest time (61–62 DAI) or pileus diameter, the 25% dose proved capable of creating an ideal nutritional equilibrium (hormesis) to convert ramie fiber substrate into high-quality fruiting body biomass.

Acknowledgments

This scientific article is intended to fulfill the requirements for the Undergraduate Thesis course in the Biology Undergraduate Program, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. The author expresses the deepest gratitude to Ruly Budiono, M.Sc., Ph.D., and Dr. Suryana, S.Si., M.P., as the supervising professors.

References

- Ananto. (2021). Supply of Coconut Water As a Nutritional Supplement CornCob Media on Growth White Oysters (*Pleurotus ostreatus*) (Vol. 3, Number 1).
- Aprilia Lestari, V., & Bagus Priambodo, T. (2020). Kajian Komposisi Lignin Dan Selulosa Dari Limbah Kayu Sisa Dekortikasi Rami Dan Cangkang Kulit Kopi Untuk Proses Gasifikasi Downdraft. *Jurnal Energi Dan Lingkungan (Enerlink)*, 16(1), 1–8. <https://doi.org/10.29122/jel.v16i1.4572>
- Branco, S., Schauster, A., Liao, H. L., & Ruytinx, J. (2022). Mechanisms of stress tolerance and their effects on the ecology and evolution of mycorrhizal fungi. In *New Phytologist* (Vol. 235, Number 6, pp. 2158–2175). John Wiley and Sons Inc. <https://doi.org/10.1111/nph.18308>
- Budiono, R., Washfanisa, H. A., Mutaqin, A. Z., Kusmoro, J., Nurzaman, M., Setiawati, T., & Hasan, R. (2024). The Growth of

- Oyster Mushroom on Ramie Chip Waste-Derived Media was Accelerated by Rice-washed Water. *Jurnal Biodjati*, 9(2), 373–386. <https://doi.org/10.15575/biodjati.v9i2.33433>
- Grich, N., Huynh, T., Kisiala, A., Palberg, D., & Emery, R. J. N. (2025). The biosynthesis and impacts of cytokinins on growth of the oyster mushroom, *Pleurotus ostreatus*. *Mycologia*, 117(1), 76–94. <https://doi.org/10.1080/00275514.2024.2401320>
- Hafizah, N., & Aspiani, A. (2016). Pengaruh Berbagai Media Tanam terhadap Pertumbuhan dan Hasil Jamur Tiram (*Pleurotus Ostreatus*). *Daun: Jurnal Ilmiah Pertanian Dan Kehutanan*, 3(1), 15–20. <https://doi.org/10.33084/daun.v3i1.156>
- Limbad, M., Gutierrez-Maddox, N., Hamid, N., Kantono, K., Liu, T., & Young, T. (2023). Microbial and Chemical Changes during Fermentation of Coconut Water Kefir Beverage. *Applied Sciences*, 13(12), 7257. <https://doi.org/10.3390/app13127257>
- Money, N. P. (2025). Physical forces supporting hyphal growth. *Fungal Genetics and Biology*, 177, 103961. <https://doi.org/10.1016/j.fgb.2025.103961>
- Mudaningrat, A., & Nada, S. (2021). Pengaruh Konsentrasi Zat Pengatur Tumbuh Dalam Kandunganair Kelapa Terhadap Pertumbuhan Tanaman Jahe (*Zingiber Officinale*) Dan Tanaman Kencur (*Kaempferia Galanga L.*). *Semnas Biologi*, 9.
- Mustaqim, W. A. (2018). Hukum Minimum Liebig - Sebuah Ulasan dan Aplikasi Dalam Biologi Kontemporer. *Bumi Lestari Journal of Environment*, 18(1), 28. <https://doi.org/10.24843/blje.2018.v18.i01.p04>
- Nurhayati, N., Dachlan, A., Yassi, A., & Tambung, A. (2025). Pertumbuhan Jamur Tiram Coklat (*Pleurotus cystidiosus*) pada Berbagai Jenis Media Tanam F1 dan Baglog. *Jurnal Agrivigor*, 169–183. <https://doi.org/10.20956/ja.v15i2.43241>
- Rosmiah, Aminah, I. S., Hawalid, H., & Dasir. (2020). Budidaya Jamur Tiram Putih (*Pluoretus Ostreatus*) Sebagai Upaya Perbaikan Gizi Dan Meningkatkan Pendapatan Keluarga. *ALTIFANI (International Journal of Community Engagement)*.
- Sumarmi. (2006). Botani Dan Tinjauan Gizi Jamur Tiram. *Jurnal Inovasi Pertanian*.
- Vigneshwaran, S., Sundarakannan, R., John, K. M., Joel Johnson, R. D., Prasath, K. A., Ajith, S., Arumugaprabu, V., & Uthayakumar, M. (2020). Recent advancement in the natural fiber polymer composites: A comprehensive review. *Journal of Cleaner Production*, 277, 124109. <https://doi.org/10.1016/j.jclepro.2020.124109>
- Widiwurjani, Retno Mulyani, I., Sa'diyah, I., & Sari, N. K. (2021). Potential Of Various Types of Media for Breeding Oyster Mushroom F2. *E3S Web of Conferences*, 328, 08015. <https://doi.org/10.1051/e3sconf/202132808015>
- Woo, S.-I., Oh, M., Lee, H. H., Song, I., Kim, S. J., Oh, Y.-L., Im, J.-H., Lee, E.-J., & Lee, M. K. (2025). Development of Hybrid *Pleurotus cystidiosus* Strains with Enhanced Functional Properties. *Foods*, 14(24), 4329. <https://doi.org/10.3390/foods14244329>