# Fungal Foams from Teak Leaves: Effect of Cold Shock and Species Variation on Growth and Mechanical Strength

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**Abstract:** Fungal foams represent sustainable alternatives to synthetic materials, yet optimization of growth and mechanical properties remains challenging. This study evaluated the effects of cold shock treatments at 0 °C for different interval days (3, 5, 7 days) on *Pleurotus* species including *P. djamor*, *P. florida*, and *P. sajor-caju* cultivated on teak leaves. Mycelial growth rates, mechanical properties (hardness, springiness, resilience), and scanning electron microscopy (SEM) were systematically evaluated. Results demonstrated that 3-day cold shock treatment consistently maximized growth, with *P. florida* (W3) achieving 1.41 cm/day. Mechanical testing revealed superior performance in 3-day treated samples: P. *florida* (W3) recorded peak hardness at 7904 g.sec, while springiness and resilience value reached 0.681 and 0.399 respectively for *P. sajor-caju* (G3) surpassing controls samples. SEM confirmed denser, thicker and intertwined hyphal networks in cold-treated samples, correlating with mechanical properties. These findings establish 3-day cold shock as an effective, non-chemical strategy to enhance fungal foam quality from agricultural residues.

**Keywords**: Fungal foam, Cold shock, Mechanical properties, Sustainable biomaterials.

## 1. INTRODUCTION

Sustainable development increasingly emphasizes the use of renewable and biodegradable materials to replace convectional plastics and synthetic foams. Agricultural residues, abundant lignocellulosic biomass derived from crop and forestry waste, present a promising feedstock for producing eco-friendly fungal foams. Utilizing such residues helps manage agricultural waste while supporting circular bioeconomy goals through conversion into value-added biomaterials with desirable properties [1]. This approach not only minimize dependence on petroleum-based materials but also promotes sustainable material innovation aligned with global environmental priorities.

Fungal species within *Pleurotus* genus (edible mushrooms) with high commercial relevance are widely cultivated particularly in Southeast Asia, due to their distinctive flavor, aroma, and notable nutritional and medical properties [2]. Besides, the demand for mushrooms in Malaysia has been steadily rising, driven by population growth and the expanding use of mushrooms in many applications including culinary practices [3]. Beyond their culinary importance, *Pleurotus* mushrooms are also well known for their strong lignocellulolytic activity and rapid mycelial growth, making them effective in converting agricultural residues into valuable biomaterials. In this study, three species were selected: *Pleurotus djamor* (pink oyster mushroom), *Pleurotus florida* (white oyster mushroom), *and Pleurotus sajor-caju* (grey oyster mushroom). Each species exhibits unique biological characteristics that support efficient mycelium development and enhance their suitability for fungal foam production.

Effective mycelial is highly influenced by the composition of the substrate and the surrounding environmental conditions. The addition of nutrient supplements such as rice bran has been shown to accelerate colonization on lignocellulosic substrates, including teak leaves, thereby enhancing foam density and mechanical performance [4]. These nutritional enhancements promote stronger hyphal bonding throughout the substrate, which supports more vigorous and uniform mycelial growth. Such improvements are especially valuable for producing bio-based materials with consistent quality and reliability.

Physical treatments are increasing explored as non-chemical approaches to enhance fungal growth and improve material performance in mycelium-based

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composites. Such treatment can modulate fungal metabolism, trigger adaptive stress response, and strengthen the mycelial network. Physical stimulation through cold shock treatment has been reported to induce stress response in fungi, triggering heat shock protein production and cell wall strengthening [5]. However, selecting an optimal cold shock temperature balances these factors, promoting growth stimulation without irreversible damage. Despite such advances, challenges remain in achieving consistent fungal foam quality due to variability in fungal strain response, substrate composition and process parameters. Addressing these challenges through controlled cold shock application could improve fungal foam uniformly and mechanical reliability [6]. In addition, existing studies mainly focus on general mycelium-based composites, while systematic investigation of cold shock treatment on fungal foam production using edible Pleurotus species and agricultural leaves residues remains limited. In particular, the combined influence of controlled cold shock and teak leaves substrate on fungal growth behavior and mechanical performance has not been sufficiently explored.

This study demonstrates that cold shock, as a non-chemical treatment, holds significant potential to enhance fungal growth and improve the resulting foam properties. By exploring teak leaves as a sustainable substrate, this study aims to determine how controlled cold shock treatment influences fungal development and mechanical improvement of produced foam. Such an approach not only offers safe alternatives to chemical enhancement but also broaden understanding of environmental stress as a tool for optimizing fungal growth. The findings from this study also provide clearer understanding of cold shock as a controllable parameter in fungal-based material production and to indicate whether it can be applied to enhance the quality and performance of fungal foams. Therefore, this study introduces cold shock as a novel and controllable process parameter for fungal foam, providing new insight into improving foam uniformly and mechanical reliability while supporting sustainable material development.

## 2. METHODOLOGY

### 2.1. Preparation of Raw Material

Agricultural residues, specifically teak leaves collected from roadside areas in Perlis, Malaysia, served as the primary substrate in this study. The leaves were cleaned to remove impurities; oven dried at 60 C to reduce moisture content and subsequently milled into fine powder to facilitate fungal colonization. To supplement nutrient availability, rice bran was incorporated as an additional growth medium component. These prepared substrates were stored under ambient conditions in sealed containers to maintain their quality before inoculation. Three species of *Pleurotus* mushrooms including *P. djamor*, *P. florida*, and *P. sajor-caju* were employed, sourced from authorized culture supplier in Beseri Agrofarm Resources, Kangar, Perlis, Malaysia.

### 2.2. Fungal Foam Formation

Fungal foam formation commenced by formulating substrate composed of teak leaves, rice brand and calcium carbonate in a weight ratio of 100:10:1, respectively following the method by Yaacob *et al*., (2023) [7]. This dry mixture was moistened using distilled water to provide an optimal environment for fungal colonization. The prepared substrate was then portioned into plastic containers, with three replicates assigned to each treatment group. These containers were sterilized using an autoclave at 120 C for 30 minutes and subsequently cooled overnight under laminar flow to prevent contamination. The sterilized substrates were inoculated with three oyster mushrooms (*Pleurotus* species) which include *P. djamor* (pink), *P. florida* (white), and *P. sajor-caju* (grey). Incubation followed under controlled conditions of 25 C temperature and 75 – 85% relative humidity and under low light intensity (10 – 15%) as adapted from Roshita *et al*., (2017) [8]. Cold shock treatments were given during fungal foam formation, and the growth phase was considered complete when the substrates were fully colonized by mycelium.

### 2.3. Cold Shock Treatment

Cold shock treatment was applied to the fungal during fungal formation by subjecting the fully colonized substrates to low temperature exposure at 0 C at different interval period of 3, 5, and 7 days. The cold-treated samples were placed in a temperature-controlled environment for the specific durations after mycelial colonization. After the cold exposure, the samples were returned to standard incubation conditions (25 C) for further development as needed. Subsequently, the treated and control fungal foams were oven-dried at 60 C for 8 hours and cooled before stored and analysis, following the method by Tan *et al*., (2024) [9] with modification. Table **2.1** summarized the experiments set of the fungal foams.

### 2.4. Analysis of Fungal Growth Rate

During the growth assessment, fungal mycelium development was recorded everyday by measuring the vertical extension of the colony within substrate-filled containers. The progression of mycelial height was plotted against time, and the fungal growth rate for each treatment was derived by applying a linear regression analysis, with the duration of incubation

**Table 2.1: Experiments Set of the Fungal Treatment**

| Fungal Species | Treatment Condition | Label |
|---|---|---|
| *P. djamor* (Pink oyster mushroom) | Control sample | PC |
| | 0 C, 3 days interval | P3 |
| | 0 C, 5 days interval | P5 |
| | 0 C, 7 days interval | P7 |
| *P. florida* (White oyster mushroom) | Control sample | WC |
| | 0 C, 3 days interval | W3 |
| | 0 C, 5 days interval | W5 |
| | 0 C, 7 days interval | W7 |
| *P. sajor-caju* (Grey oyster mushroom) | Control sample | GC |
| | 0 C, 3 days interval | G3 |
| | 0 C, 5 days interval | G5 |
| | 0 C, 7 days interval | G7 |

(days) as the independent variable and the measured colony height (cm) as the dependent variable. The procedure allowed for a systematic quantification of growth dynamic over the entire colonization period. Such a methodological approach is consistent with standard protocols utilized in fungal monitoring and rate determination in solid substrates [10].

### 2.5. Mechanical Properties of Fungal Foam

The mechanical properties of fungal foams, specifically hardness, springiness and resilience, were evaluated using a Stable Micro Systems TA.XT2 texture analyzer. Cylindrical samples of the foam underwent testing via the double bite compression method using cylindrical probe (P/75), which stimulates repeated compression forces at a constant speed of 5 mm/s. This double compression approach also known as Texture Profile Analysis (TPA), provides detailed insights into the material's hardness, springiness and resilience, which is the key in determining the suitability of foam for industrial applications [11]. This method was performed following Bourne's (1978) [12] and adapted for foam materials by [13] Sari & Rafisa (2023). The testing parameters employed are detained in Table **2.2**.

**Table 2.2: Setting for Texture Analyzer**

| Test Mode | Compression Mode |
|---|---|
| Pre-test speed | 10 mm/s |
| Test speed | 5 mm/s |
| Post-test speed | 20 mm/s |
| Target mode | Strain 10% |
| Count | 2 |

### 2.6. Scanning Electron Microscopy

The surface morphology of the fungal foam samples was analyzed using a JEOL JSM-6390LV scanning electron microscope (SEM). To prevent the buildup of electrostatic charge during imaging, the chosen samples (control and treated) were coated with thin layer of platinum.

## 3. RESULTS AND DISCUSSION

### 3.1. Growth Performance of Fungal

In this study, the influence of cold shock on the growth behavior of different oyster mushroom species on teak leaves was investigated. Figure **3.1** presents the mycelial growth rates for each species under control condition (PC, WC, GC) and after treated with cold shock at 0 C for 3 days (P3, W3, G3,) 5 days (P5, W5, G5) and 7 days (P7, W7, G7). Among these treatments, the 3-days interval cold exposure consistently produced the highest growth rate for all fungal species including *P. djamor* (P3) at 1.19 cm/day. *P. florida* (W3) at 1.41 cm/day and *P. sajor-caju* (G3) at 1.36 cm/day. However, extending cold shock period to 5 days and 7 days did not give further improvement, and some showed slightly reduced growth.

This pattern suggests that a short interval of cold shock is optimal for stimulating mycelial expansion, while longer exposure may start to limit growth rather that enhance it, which in line with reports that appropriately timed cold shock can accelerate *Pleurotus* mycelium development and shorten colonization time [14]. In addition, these findings indicate that the response of the oyster mushroom mycelium to cold shock depends on the duration of

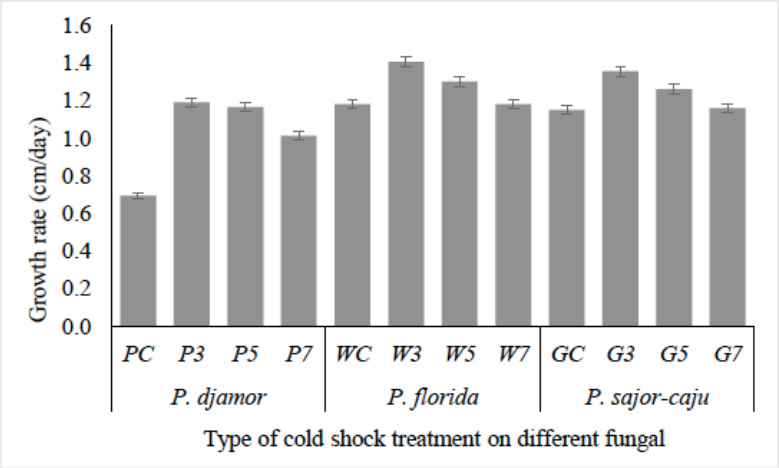


**Figure 3.1:** Mycelial growth rate on different cold chock treatments.

exposure. A short cold shock interval appears to activate protective stress pathway including increased production of cold-responsive and heat-shock proteins such as HSP70 and HSP90, which help stabilize cell structure and support faster growth [15]. In contrast, when the low-temperature treatment is prolonged, metabolic activity is likely to decreased and additional stresses such as membrane rigidity can occur, leading to slower growth rate of fungal. Similar duration-dependent adaptations have been reported for fungi, where low-temperature exposure induces distinct sets of stress-response proteins that support cold environment [16].

### 3.2. Mechanical Properties

#### *3.2.1. Hardness*

Hardness is a key indicator of mechanical strength of fungal foams. Higher hardness typically reflects denser mycelial compaction and stronger internal bonding. Figure **3.2** shows hardness of fungal foams responded to the duration of cold shock treatment across all fungal species. For *P. florida*, the 3 days interval (W3) showed highest hardness (7904 g.sec) across all type of samples. Similarly, *P. djamor* (P3) and *P. sajor-caju* (G3) exhibited higher hardness at 7504 g.sec and 7733 g.sec respectively, for 3-day treatment compared to respective controls (PC, GC) at 3250 g.sec and 4073 g.sec respectively.

This suggests that short-term cold exposure stimulates a strengthened mycelial network, possibly due to increased chitin and β-glucan cross-linking during recovery from stress. Majib *et al*. (2023) [17] reported that compact mycelial networks yield greater hardness in *Pleurotus* foams, supporting these findings. Conversely, extended cold exposure (5-7 days; P5, P7, W5, W7, G5, G7) resulted in reduced hardness (6365 g.sec to 6921 g.sec) across the species, suggesting excessive stress may lead to weakened network rigidity due to structural damage. These findings are consistent with Madusanka *et al*. (2024) [18], who highlighted that prolonged stress can weakened hyphal cohesion and reduce mechanical performance. Therefore, cold treatment for three days appears optimal for enhancing hardness.

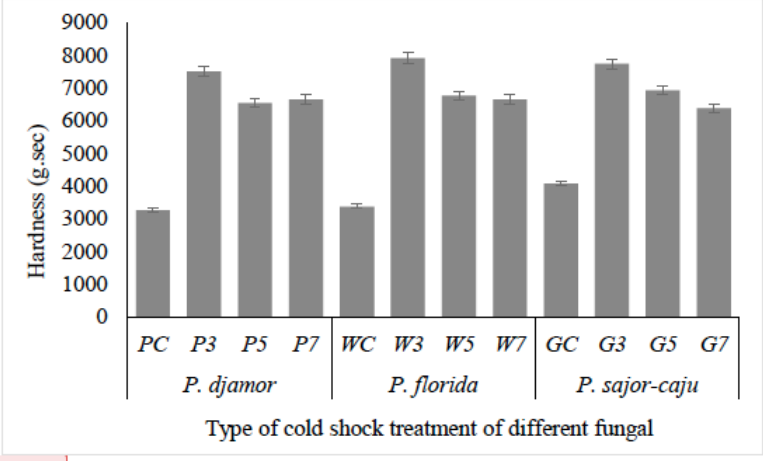


**Figure 3.2:** Hardness of fungal foam produced from different cold shock treatments.

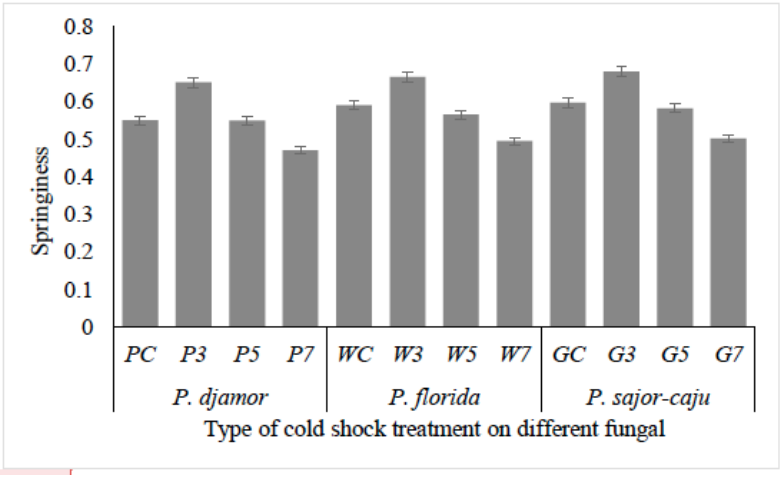


**Figure 3.3:** Springiness of fungal foam produced from different cold shock treatments.

### *3.2.2. Springiness*

Springiness represents the ability of fungal foams to return to their original height after compression. This property is important parameter for shock-absorbing applications. Figure **3.3** shows that samples that treated with cold shock for 3 days; P3, W3 and G3 exhibited highest springiness at 0.652, 0.667, 0.681 respectively. Among all species, *P. sajor-caju* treated for 3 days (G3) demonstrated the greatest elastic response, followed by *P. florida* (W3).

Longer cold shock duration (5 and 7 days) resulted in reduced springiness, indicating potential structural weakening due to extended cold exposure. This confirms a balance between rigidity and elasticity, as excessive rigidity may limit flexibility. Similar trends observed by Madusanka *et al*. (2024) [18] and Chen *et al*. (2025) [19], where optimized environmental conditions enhanced elastic properties by promoting cohesive network structuring without inducing cellular damage.

### *3.2.3. Resilience*

Resilience defines the ability of foams to absorb and release energy during compression, indicating structural recovery efficiency and resistance to permanent deformation[20]. As illustrated in Figure **3.4**, cold shock treatment for 3 days (P3, W3, and G3) yielded the highest resilience values across all species at 0.386, 0.397. 0.399, surpassing the control samples at 0.313 (PC), 0.343 (WC), and 0.326 (GC). This explains, shorter duration of treatment has great recovery ability. When treatment duration was extended to 5 and 7 days, resilience values of P5, P7, W5, W7, G5, G7 were declined at range of 0.313 to 0.356. This trend mirrored in hardness and springiness, suggests that overly prolonged cold exposure may disrupt cellular processes.

## 3.3. Morphological Characterization

Figure **3.5** compares the surface morphology of *P. florida* fungal foams for the control (WC) and the best cold shock treated sample (W3). Since the growth rate and mechanical properties of the three oyster species

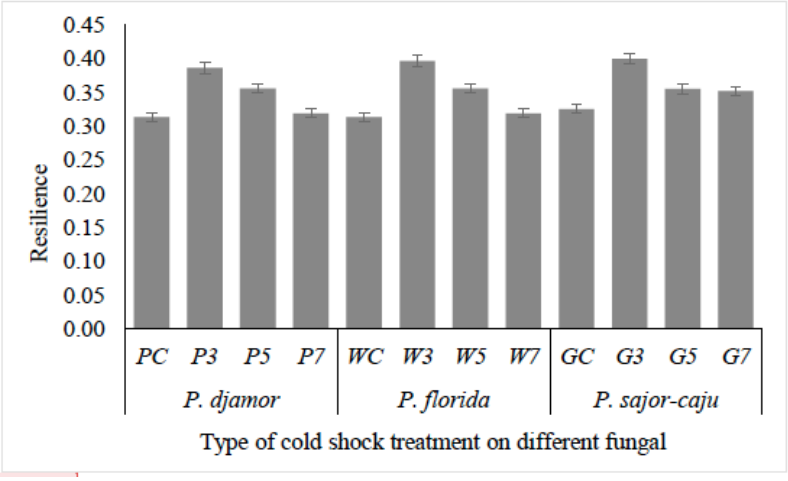


**Figure 3.4:** Resilience of fungal foam produced from different cold shock treatments.

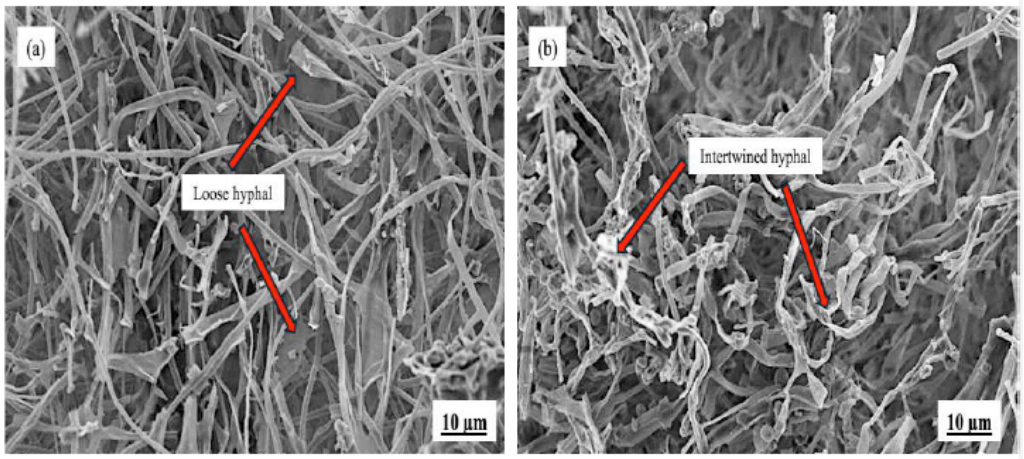


**Figure 3.5:** SEM micrograph of fungal foam (**a**) control samples (**b**) cold shock treated samples

were not statistically significant different, *P. florida* was selected as a representative species for detailed SEM observation because of the promising growth performance as mycelium-based material.

In WC sample (Figure **3.5a**), the hyphal network is relatively loose and open, which is usually linked to the lower strength, springiness and resilience [21]. Contrary to the cold-treated sample of W3 (Figure **3.5b**), the micrograph shows a denser and more thicker hyphae. There is also formation of intertwined hyphae can be spotted in the figure. This compact structure is consistent with the cold-induced stress responses that reinforce the cell wall and promote tighter hyphal [22] (Boey *et al*., 2022), and it agrees with the higher hardness, resilience and springiness measured for W3.

## CONCLUSION

This study revealed the significant potential of fungal foams derived from teak leaves through cultivation of Pleurotus species under cold shock treatments. Cold exposure at 3-days interval consistently enhanced mycelial growth, with *P. florida* (W3) demonstrating the fastest growth rate approximately 1.41 cm/day, supporting its suitability for efficient fungal foam production. Besides, mechanically, this treatment increased hardness markedly, with *P. florida* samples (W3) displaying value of 7904 g.sec, emphasizing improved structural properties. Elastic recovery, measured through springiness, was notably elevated across all species under 3-day cold shock, with *P. sajor-caju* (G3) exhibiting highest springiness at 0.681 g.sec. Similarly, resilience testing that reflect energy recovery was optimized at this interval, with all species demonstrating enhanced performance compared to prolonged intervals and control samples. Microscopic analysis of cold shock treatment samples revealed denser and thicker hyphal network, which directly contributed to the mechanical enhancements. These findings suggest that controlled cold chock at specific intervals effectively stimulates fungal physiological response, resulting in better growth and enhance mechanical properties of fungal foam produced.

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## CONFLICTS OF INTEREST

The authors have no disclosure to declare

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