

## Influence of wood species on the physical and mechanical performance of *Ganoderma lucidum*-based mycelium composites for sustainable packaging applications

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### Abstract:

This study addresses the need for a clearer understanding of how specific lignocellulosic substrates influence the performance of mycelium-based composites. Mycelium-based composites were prepared by combining *Ganoderma lucidum* (Curtis) mycelium with sawdust from *Quercus sp.* (oak) and *Fagus orientalis* (oriental beech) using a molding process. The aim was to evaluate the effects of wood species on the physical, thermal, and mechanical properties of these composites under controlled conditions. Physical properties (density), thermal stability (thermogravimetric analysis, TGA), and mechanical performance (compressive strength) were evaluated, and morphological characteristics were analyzed using scanning electron microscopy (SEM).

The results showed that substrate type significantly influences composite performance. Oak-*Ganoderma lucidum* (curtis) composites exhibited superior physical and mechanical properties compared with beech-*Ganoderma lucidum* (curtis) composites, with a density of 239 kg/m<sup>3</sup> and a compressive strength of 0.20 MPa. SEM analysis revealed a filamentous network of tubular hyphae of varying diameters surrounding the wood cell walls, while TGA results indicated similar thermal degradation behavior for both composites.

The developed mycelium-based composites exhibited properties comparable to expanded polystyrene (EPS), highlighting their potential as sustainable alternatives for packaging and insulation. Overall, this study demonstrates that substrate composition plays a governing role in determining material performance and informs the design of bio-based composites.

**Keywords:** Bio-based materials, *Ganoderma lucidum*, lignocellulosic substrate, mechanical properties, mycelium-based composites, wood sawdust.

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## Introduction

Recent environmental pollution and raw material depletion have spurred the development of green approaches, leading to increased interest in sustainability and ecological design. Advances in material innovation and design, enabled by experimental methods, have facilitated the production of bio-based materials. Researchers are incorporating biological studies and biomimicry into their designs, aiming to create eco-friendly solutions that reduce the harmful effects of synthetic-based products (Alici and Dalkılıç 2022). Furthermore, rising global agricultural production generates significant amounts of byproducts and waste, including cottonseed hulls, rice straw, sugarcane bagasse, coconut coir pith, sawdust, and corn stalks. Transforming agricultural waste into products enables more efficient resource use and converts it into valuable raw materials as part of a circular economy model (Peng *et al.* 2023). Utilizing various agricultural wastes reduces dependence on natural resources and fosters the development of renewable, eco-friendly materials. This presents a significant alternative for producing materials used in construction, packaging, textiles, and bioplastics.

Expanded polystyrene (EPS) foam is widely used in packaging and insulation applications (Xie *et al.* 2022, Rajendran 2022, Li *et al.* 2025). It plays a crucial role in safely transporting white goods, electronic components, and fragile materials and enhancing building energy efficiency. However, EPS poses significant risks to human and environmental health, as it is derived from petroleum-based materials that cause air and environmental pollution during production. (Bruscato *et al.* 2019, Ozen *et al.* 2021, Yildirim *et al.* 2022, Polruang *et al.* 2025). EPS foams are not biodegradable and are resistant to photolysis (Bandyopadhyay & Basak, 2007). When exposed to sunlight, rain, and ocean water (especially in tropical waters), they can decompose into their components, including styrene monomers (2), which are classified as possible human carcinogens (3).

Styrene trimers can increase thyroid hormone levels (Yanagiba *et al.* 2008). Styrene exposure has an adverse effect on human health. Exposure to residual monomers, dimers, and trimers is directly linked to reproductive abnormalities (Swan *et al.* 2005). Various studies have reported neurological disorders and a central nervous system depressant role (Murata *et al.* 1991, Cohen *et al.* 2002). Long-term exposure to styrene can affect human health by causing dermatitis and irritation of the eyes, throat, and respiratory tract (Arora and Manila 2021). Polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyamide (PA) offer benefits such as low thermal conductivity and high mechanical strength. Nevertheless, their use is restricted due to disadvantages like low thermal stability, high flammability, and rapid combustion (Sorrentino *et al.* 2007, Ergün *et al.* 2024).

Mycelium, the vegetative structure of filamentous fungi, comprises networks called hyphae (Fricker *et al.* 2007). The hyphae act as natural binders by breaking down and adhering to the surfaces of organic materials under specific ambient conditions, without requiring additional energy. Fungal hyphae can bind cellulose fibers, especially lignin, enabling the formation of lightweight, bio-based materials in various forms without synthetic binders (Attias *et al.* 2020, Abhijith *et al.* 2018). Due to these characteristics, mycelium bio-composites have been studied. *Ganoderma lucidum* (Curtis) P. Karst., *Pleurotus ostreatus* (Jacq.) P. Kumm. and *Trametes versicolor* (L.) Lloyd is one of the most commonly utilized species for producing mycelial bio-composites (Sydor *et al.* 2022, Jones *et al.* 2018, Attias *et al.* 2020). This is mainly due to their high colonization rates and their capacity to utilize various woody plant biomasses. These fungi are saprotrophic, breaking down wood and leaving behind whitish fibrous debris, they are called white rot fungi. They belong to the Basidiomycota phylum and the Agaricomycetes class and constitute an important group within the phylum, comprising approximately 30-32 % of fungal diversity and containing an estimated 30000 different species (Pinar and Rodríguez-Couto 2024). They can break down all components of the plant cell wall through various mechanisms,

including extracellular enzymatic processes and non-enzymatic processes such as reactive oxygen species (Kijpornyongpan *et al.* 2022). The main extracellular enzymes are lignin peroxidases (LiPs, EC 1.11.1.14), manganese-dependent peroxidases (MnPs, EC 1.11.1.13), and laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3) and oxidase-producing peroxidases and mycelium-associated dehydrogenases. (Martínez *et al.* 2005).

These enable white rot fungi to break down complex plant cell wall polymers, such as cellulose, hemicellulose and lignin. White rot fungi make an important contribution to the ecosystem by effectively breaking down lignin into carbon dioxide (CO<sub>2</sub>) and water (H<sub>2</sub>O) (Floudas *et al.* 2012, Kundu 2021, Llanos-López *et al.* 2023). White rot fungi can also neutralize the toxicity of aromatic compounds, such as terpenes (Elsacker *et al.* 2021). These fungi are particularly effective at degrading lignin due to the production of ligninolytic extracellular oxidative enzymes. The most common white rot fungi used in lignocellulose processing include *Phanerochaete chrysosporium*, *Phanerochaete carnosa*, *Ganoderma lucidum*, *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Phanerochaete chrysosporium*, and *Trametes sp* (Vicuña 2000).

Previous studies have shown that mycelium-based composites can be produced from a wide range of lignocellulosic substrates, including sawdust, agricultural residues, and natural fibers, yielding materials with diverse physical and mechanical properties (Jones *et al.* 2018, Joshi *et al.* 2020, Sivaprasad *et al.* 2021, Peng *et al.* 2023). However, these studies also report substantial variability in density, mechanical strength, and thermal behavior across substrates.

Although this suggests that substrate composition is critical to material performance, the relationship between substrate type and composite properties has not been systematically evaluated. This lack of understanding limits the ability to design and optimize mycelium-based materials for specific applications. The density and mechanical performance of mycelium-based composites vary significantly with processing conditions (Shakir *et al.* 2020, Chan *et al.*

2021, Manan *et al.* 2021), fungal species (Bruscato *et al.* 2019) and substrate type (Appels *et al.* 2018), yielding materials ranging from low-density, foam-like structures to denser composites with enhanced mechanical resistance. However, the specific role of substrate composition as a governing factor in determining performance remains insufficiently understood.

This study aims to evaluate how wood species (oak and beech sawdust) influence the physical, mechanical, thermal, and morphological properties of *Ganoderma lucidum*-based mycelium bio-composites, to determine how substrate composition governs material performance and suitability for packaging and insulation applications.

## **Materials and methods**

### **Materials**

Sawdust from oak (*Quercus sp.*) and oriental beech (*Fagus orientalis* L.) were collected from timber and furniture factories in Muğla, Türkiye. Beech sawdust was chosen for its widespread distribution in Türkiye and its significant use in the furniture industry. Oak is also commonly utilized in this sector. Sawdust from these species is plentiful and readily available. Specimens of *Ganoderma lucidum* (Curtis) P. Karst. were collected in July 2024 from Anatolian sweetgum

(*Liquidambar orientalis* Miller), an endemic species in Turkey's flora, located in the Fethiye district of Muğla. Following the taxonomic identification of the sporocarps (Phillips 1981, Hall *et al.* 2003) the specimens were preserved in the Fungarium of the Department of Biology, Faculty of Science, Muğla Sıtkı Koçman University, and assigned a catalog number. One of the morphologically identified fruit bodies was set aside for isolation.

## **Isolation and preparation of *Ganoderma lucidum* (Curtis) P. Karst**

### **Mycelium**

The surface of one of the selected fruitbodies was cleaned of visible contaminants using a brush. Next 0,3-0,5 cm pieces were aseptically cut from the inner core of the fruit body using forceps and a scalpel, and sterilized by spraying with 98 % ethanol. Four to six small tissue inoculations were made in each petri dish (90 x 15 mm) prepared by adding 0,15 g/L thiamine (Sigma Aldrich®, Cas Number: 67-03-8) and 0,50 g/L streptomycin (Sigma Aldrich®, Cas Number: 3810-74-0) to sterile Potato Dextrose Agar (Sigma Aldrich®, Cas Number: 3810-74-0) medium and left for incubation at  $28 \pm 0,5$  °C. After five to seven days the mycelia that showed homogeneous development in the Petri dishes were transferred to fresh PDA for purification and incubated under the same conditions. Once the fungus completely covered the agar surface, it was stored at +4 °C for later use.

A mixture (mass/mass) of wheat grain (95 %), gypsum (4 %), and CaCO<sub>3</sub> (1 %) was moistened to 60 % (mass/mass), placed into 500 ml plastic bottles at a 4/3 ratio (Beje *et al.* 2013), sealed with cotton, wrapped in aluminum foil, and autoclaved at 121°C for 30 minutes. The mixture was allowed to cool overnight, and 5 mm square pieces of agar with mycelium were inoculated

into flasks and incubated at  $28 \pm 0,5$  °C for 13 days. At the end of this period, the wheat grains were completely covered with mycelium (spawn). The prepared spawn was stored at +4 °C for use later.

### **Preparation of the mycelium bio-composites**

Wheat bran, oak, and beech sawdust obtained from factories were oven-dried at 70 °C for 2 hours to reduce the moisture content to around 10 % to minimize contamination. To promote mycelial growth, 10 % (w/w) wheat bran and 2 % (w/w) CaCO<sub>3</sub> were added to each substrate to raise the pH level. The oven-dried oak and beech sawdust were sieved using a vibrating sieve shaker to remove particles larger than 2 mm. Oak and beech sawdust were then mixed separately with dry wheat bran (10 % w/w), and CaCO<sub>3</sub> (2 % w/w) (Chan *et al.* 2021), and water was added to both mixtures to achieve a moisture content of 60 %. The prepared oak and beech mixtures were placed into autoclavable bags (approximately 2 liters) and autoclaved twice at 121 °C for 30 minutes separately.

After autoclaving, the bags were cooled overnight at room temperature. Then, 3 % (w/w) spawn was added to the mixtures and thoroughly mixed. The substrate was then aseptically transferred to glass molds (200 × 30 mm) using a sterilized spatula. Pressure was applied to the surface to compact the substrate, and the lids of the glass molds were closed. The molds were wrapped in parafilm to maintain moisture balance and minimize contamination, and then incubated under the previously described conditions. The development of the substrates placed into a total of 10 glass molds was observed every 2-3 days. According to Jiang *et al.* (2013), extending the mycelium development period leads to a smoother substrate surface, while Shakir *et al.* (2020)

indicated that prolonging incubation time did not impact the mechanical properties of the final material. To ensure uniform and consistent coverage of the substrate surfaces, mycelial growth was terminated after 18-21 days (Aquino *et al.* 2022), depending on the substrate type (oak or beech sawdust). Once mycelial growth was completed, each mold was oven-dried at 65 °C for 2-3 days to a stable mass.

### **Performance evaluation of the mycelium bio-composites**

The prepared mycelium bio-composite samples oak sawdust-*Ganoderma lucidum* (O-GL) and beech sawdust-*Ganoderma lucidum* (B-GL) were cut to dimensions specified in the characterization standards after being taken out of the molds. They were then stored in a climate chamber at a relative humidity of 65 % and a temperature of 20 °C until they reached equilibrium moisture content. The physical properties (density, moisture content), thermal properties (thermal stability via thermogravimetric analysis), and mechanical properties (compressive strength) of the mycelium bio-composites were evaluated. Morphological structure was analyzed using a scanning electron microscope (SEM). The results were also compared and discussed with relevant studies. The characterization list of the developed mycelium composites is given in Table 1.

**Table 1:** Characterization list of the developed mycelium composites.

Characterization	Standard	Sample Dimensions
Density	ASTM C303 (2016)	50 mm × 50 mm × 18 mm
Moisture Content	TS EN 322 (1999)	50 mm × 50 mm × 18 mm
Thermogravimetric Analysis	-	6-10 mg powder
SEM	-	-
Compressive Strength	ASTM C 165-07 (2017)	55 × 55 × 15 mm

### Density

The density of the mycelium bio-composites was determined according to ASTM C303 (2016). Ten samples were prepared for each group (50 mm × 50 mm × 18 mm). The density was calculated by dividing the measured mass ( $m$ , g) by the measured volume ( $v$ , cm<sup>3</sup>) using the formula (Equation 1).

$$d = \frac{m}{v} \quad (1)$$

### Moisture content

Moisture content of the mycelium composites was determined according to the TS EN 322 (1999) standard. Five samples from each group were prepared, (50 mm × 50 mm × 18 mm). The samples were weighed and dried at  $103 \pm 2$  °C until reaching a constant mass. During

measurements taken at 6-hour intervals, the mass difference between consecutive weighings was deemed negligible if it was less than 0,01 g, indicating a constant mass. After drying, the samples were removed from the oven. The moisture content values were then calculated using the formula (Equation 2).

$$Mc = \frac{m_r - m_0}{m_0} \times 100 \quad (2)$$

Mc=Moisture content (%)

M<sub>1</sub>= Moist weight (g)

M<sub>0</sub>= Dry weight (g)

### **Morphological analysis**

The morphological structure of the mycelium bio-composites was examined using a JSM-7600F scanning electron microscope (SEM) at the Çukurova University Central Laboratories in Adana, Türkiye. The samples (non-conductive) were coated with a thin layer of gold Au/Pd (~5-10 nm) with a sputter coater and placed in the slots. The images were captured with an acceleration voltage of 20 kV. The images were magnified at 500x and 2000x.

### **Thermogravimetric analysis (TGA)**

The mycelium bio-composite samples from each group were grounded into powder and then subjected to TGA. The biological duplicates of six- to ten-mg samples were analyzed. The samples were initially held at 30 °C for 1 minute, then heated to 600 °C at 5 °C/min. Finally, the samples were maintained at 650 °C for one minute, and the results were recorded. The TGA was conducted using a PerkinElmer TGA 4000 device in the Department of Chemistry at Muğla Sıtkı Koçman University.

### **Compressive strength**

The compressive strength of the mycelium bio-composites was measured according to ASTM C165-07 (2017) using a universal testing machine at the Faculty of Technology, Muğla Sıtkı Koçman University. Ten samples from each group were prepared, and the testing speed was set to 1 mm/min. Compressive strength was defined as the stress at 20% deformation, a commonly used criterion for assessing the mechanical performance of low-density packaging materials.. The compressive resistance was calculated as shown in Equation 4.

$$S = \frac{W}{A} \quad (4)$$

S: Compressive Strength (MPa)

W: Load at 20% deformation (N)

A: Area of the tested sample (mm<sup>2</sup>)

## Statistical analysis

Density and compressive resistance data were analyzed using one-way ANOVA to assess statistically significant differences ( $\alpha = 0.01$ ). When significant effects were detected, pairwise comparisons among groups were conducted using the Tukey–Kramer Honestly Significant Difference (HSD) test ( $\alpha = 0.05$ ). A stricter significance level for ANOVA ensured a more conservative overall test, while the Tukey–Kramer HSD test at  $\alpha = 0.05$  enabled detailed multiple comparisons among group means. Statistical analyses and graphs were generated using OriginLab Pro 2024 (OriginLab Co., New Castle, DE, USA).

## Results and discussion

### Density and moisture content

The density and moisture content results of the mycelium bio-composites, along with their statistical analysis, are shown in Table 2.

**Table 2:** The density and moisture content of the mycelium bio-composites.

Group	Density (kg/m <sup>3</sup> )	Moisture Content (%)
B-GL	188 (15,21) A	11,12 (15,9) A
O-GL	239 (16,04) B	10 (20,2) A

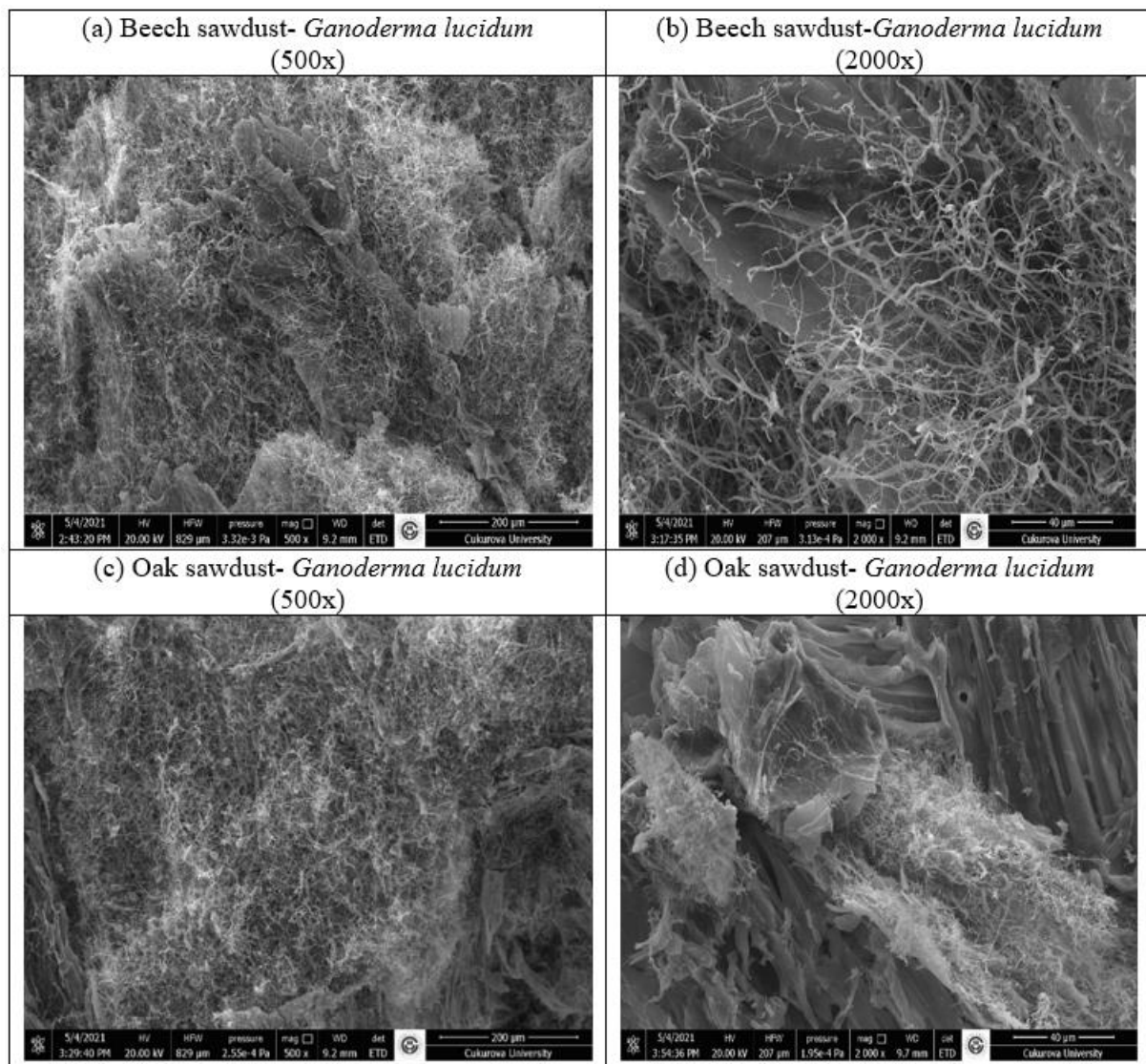
Values in the parentheses indicate the coefficient of variation (%COV); A and B letters indicate the significant differences between the groups.

The B-GL density was significantly lower than O-GL mycelium bio-composites. The observed differences in density values can be attributed to both the substrate's intrinsic density and the extent of mycelium colonization, both of which are expected to influence the mechanical performance of the composites. There is often a positive correlation between density and mechanical properties, with increasing material density leading to significant improvements in compressive strength, elastic modulus, and modulus of rupture. (Sun *et al.* 2020; Peng *et al.* 2023). However, when a biological process is involved, a different mechanism also comes into play. As the mycelium consumes the substrate, the overall material density may decrease; however, over time, improvements in mechanical properties can be observed as the mycelium network develops, forming a strong binding structure (Soh *et al.* 2020).

The moisture content of B-GL and O-GL bio-composites was measured at 11,12 % and 10 % respectively. It showed that there are no significant differences across the samples, so the influence on the thermal and mechanical properties is expected to be limited or nonexistent. Different letters (A, B) indicate statistically significant differences between groups according to the Tukey–Kramer HSD test ( $p < 0.05$ ), with groups sharing the same letter not being significantly different.

## **Morphology**

The morphological structure of the prepared mycelium bio-composites was examined using a scanning electron microscope (SEM). SEM images of the mycelium bio-composite samples are shown in Figure 1.



**Figure 1:** SEM images of the mycelium bio-composites.

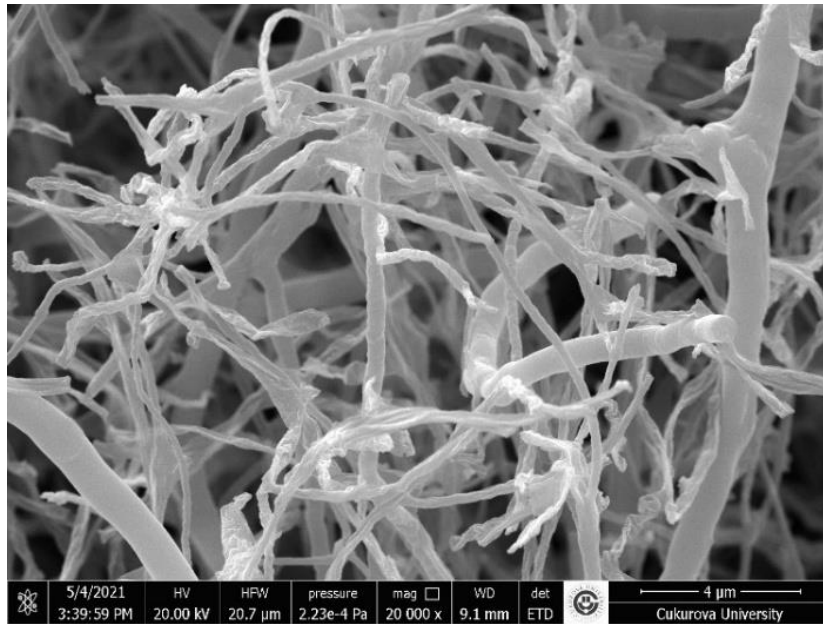
SEM images reveal that mycelium bio-composites exhibit complex surface topography. In the early stages of wood decay, fungal hyphae penetrate the wood, initiate colonization, and secrete enzymes that alter the substrate's color (Zabel and Morrell 2020). As illustrated in the SEM images (Figure 1), the fungal hyphae create a fibrous network that envelops the wood cell walls. The *Ganoderma lucidum* hyphae colonize beech sawdust in a patchy manner, with some areas showing sparse colonization and others displaying denser growth (Figure 1a). In contrast, the colonization around oak sawdust is more uniform and denser (Figure 1c). These observations

indicate that oak sawdust provides a more conducive environment for the growth of *Ganoderma lucidum* mycelium, likely due to its higher lignin content.

Studies have shown that lignin plays a major role in mycelium development and fruiting body formation due to the degradation of lignin by enzymes such as hemicellulose and manganese peroxidase produced by *Ganoderma lucidum* from different lignocellulose-containing substrates (Haisheng *et al.* 2017, Sudheer *et al.* 2018, Atila 2020). Additionally, certain production parameters, such as insufficient airflow, can affect mycelial colonization (Dias *et al.* 2021). It is believed that this led to greater degradation and affected the physical and mechanical properties of the prepared mycelium bio-composites.

The formation of a homogeneous, dense mycelial network around substrate particles (as observed in SEM images, where the particles are completely covered by hyphae) improves mechanical performance by strengthening the bond between the hyphae and the substrate, as supported by Sun *et al.* (2019) and de Lima *et al.* (2020). Furthermore, the reduction in the interconnection length between hyphae observed in SEM analyses indicates a denser network structure, a condition associated with higher mechanical resistance (Appels *et al.* 2019). In brief, it is understood that the tight, interconnected hyphal networks observed via SEM improve the mechanical properties of composites by enabling more effective load transfer between the substrate and the mycelium.

The *Ganoderma lucidum* mycelium hyphae were imaged at 20000 x magnification, as shown in Figure 2. It can be observed that *Ganoderma lucidum* mycelium consists of tubular hyphae of varying diameters and sizes ( $\mu\text{m}$ ) and is arranged in a filamentous network.



**Figure 2:** SEM images of the pure *Ganoderma lucidum* mycelium hyphae.

### **Thermal stability**

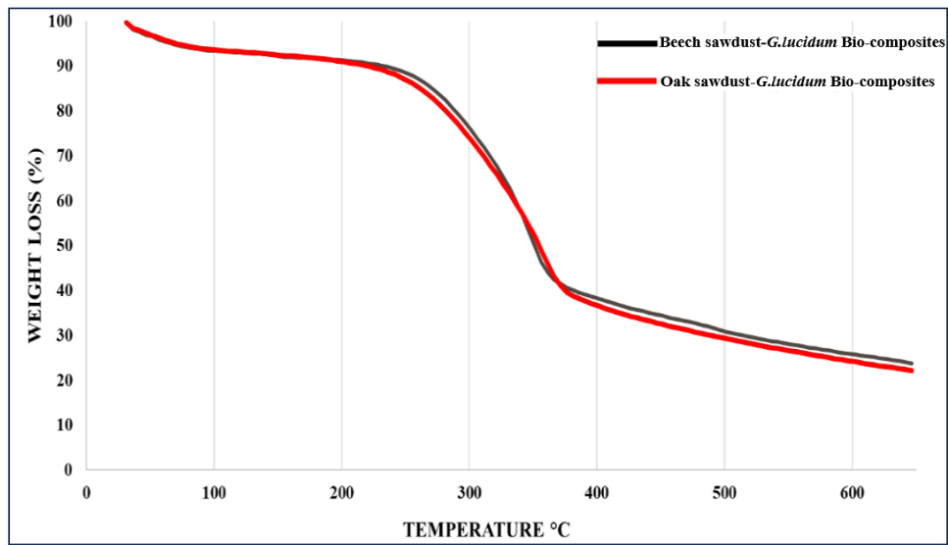
The thermal stability of the prepared mycelium bio-composites was analyzed using a thermogravimetric analyzer. The onset decomposition temperature ( $T_{\text{onset}}$ ), the maximum decomposition temperature ( $T_{\text{max}}$ ), and the residue amount of the mycelium bio-composites recorded as summarized in Table 3 and illustrated in Figure 3.

**Table 3:** The thermal properties of mycelium bio-composites.

Mycelium Bio-composites	Tonset (C°)	Tmax (C°)	Residue (%)
B-GL	251,5	351,80	23,76
O-GL	240,5	350,08	22,10
Pure mycelium (Sun <i>et al.</i> 2019)	208	-	-
Raw beech wood (Marková <i>et al.</i> 2018)	220	330	-
Raw oak wood (Marková <i>et al.</i> 2018)	240	310-320	-

TGA curves of the prepared mycelium bio-composites show closely similar Tonset, Tmax, and residue values (Figure 3). The TGA graph consists of three distinct sections outlined by Jones *et al.* (2018). Water evaporates in the initial temperature range of 35-100 °C. In the second section, between 200 and 375 °C, it can be inferred that the wood chip and mycelium structural components, such as amino acids and chitin, degrade rapidly. The third and final section addresses the volatile matter, which burns at a higher temperature of 450-600 °C due to air combustion.

In TGA analyses, the chemical structure of the primary substrate plays a decisive role in the overall thermal decomposition profile of mycelium composites (Jones *et al.* 2018; de Lima *et al.* 2020). Because beech and oak are hardwood species with similar chemical compositions, the thermal degradation behaviors ( $T_{\text{onset}}$  and  $T_{\text{max}}$ ) of composites produced from these raw materials are very similar. Indeed, in the study conducted by Sun *et al.* (2019), the initial decomposition temperatures of the wood (243.8 °C,  $T_{\text{onset}}$ ) and wood–mycelium (241.9 °C,  $T_{\text{onset}}$ ) were similar, as were the peak decomposition temperatures ( $T_{\text{p1}}$  and  $T_{\text{p2}}$ ). This indicates that mycelium has a limited effect on the thermal stability of wood-based substrates.



**Figure 3:** TGA curves of the mycelium bio-composites

### Compressive strength

The compressive strength values of the prepared mycelium bio-composites, commercial EPS foams, and mycelium bio-composites from various literature studies are presented in Table 4.

**Table 4:** The compressive strength of the mycelium bio-composites.

Mycelium Bio-composites and Relevant Studies	Description of the produced materials	Compressive Resistance (MPa)	Density (kg/m <sup>3</sup> )
B-GL	Beech sawdust <i>Ganoderma lucidum</i> by the casting method for packaging	0,12 (14,15) A	188 (15,21) A
O-GL Oak sawdust- <i>Ganoderma lucidum</i>	Oak sawdust- <i>Ganoderma lucidum</i> by the casting method for packaging	0,20 (9,17) B	239 (16,04) B
EPS Vidholdová <i>et al.</i> (2019)	Commercial petroleum-based EPS foam	0,096	13,50 – 18
Commercial EPS Castiglioni <i>et al.</i> (2017)	Commercial petroleum-based EPS foam	0,135	200
Vidholdová <i>et al.</i> (2019)	Wood chipped particles for the manufacturing of commercial particle boards by using the growth activity of the fungus <i>Trametes versicolor</i>	0,023-0,199	103-189
Holt <i>et al.</i> (2012)	The blends were comprised of processed CPB, cottonseed hulls, starch, and gypsum. The best results revealed blends that met or exceeded the characteristics of extruded polystyrene foam.	0,001-0,072	-
de Lima <i>et al.</i> (2020)	This work evaluated the effect of peach-palm residues as substrate for the growth of mycelium based on <i>Lentinula edodes</i> . They were also supplemented with cassava bran and various sources of nitrogen, ammonium sulfate, potassium nitrate, and soy flour, to analyze their effects on the physicochemical, enzymatic activities, and thermal and mechanical properties of the final composite.	0,7-0,15	
Sivaprasad <i>et al.</i> (2021)	Mycelia from the fungi, <i>Pleurotus Ostreatus</i> , are used and grown on the sawdust-coir pith substrate. They produced EPS like bio-composite material.	0,03-0,4	
Yang <i>et al.</i> (2017)	Three different mixing protocols with various substrate materials, including wood pulp, millet grain, wheat bran, a natural fiber, and calcium sulfate, and two packing conditions were tested. They reported that these biofoams offer great potential for application as an alternative insulation material.	0,35-0,57	-
Peng <i>et al.</i> (2023)	Mycelium bio-composites were developed by incubating <i>Pleurotus ostreatus</i> on different substrates derived from agricultural byproducts, including rice straw, bagasse, coir pith, sawdust, and corn straw. They reported that these mycelium bio-composites offer great potential for application as an alternative packaging material.	0,456-270	249-336
Biby <i>et al.</i> (2025)	This study compared their performance with that of conventional foams on substrates such as sawdust, cardboard, paper, cocopith, and hay. Mycelium of <i>Ganoderma L.</i> and <i>Pleurotus Pstreatus</i> were used.	2,72-0,42	-

Values in the parentheses indicate the coefficient of variation (COV); A and B letters indicate the significant differences between the groups. EPS: Expanded polystyrene foam

In mycelium-based composites, density and mechanical strength are directly and positively correlated. Increasing density yields significant improvements in mechanical performance, as reported by several studies (Zhang *et al.* 2017; Sun *et al.* 2020; Peng *et al.* 2023). As density increases, porosity decreases, while bending strength and the modulus of elasticity increase. Furthermore, as the inter-hyphal connection distance decreases, a stiffer network forms, and the material transitions from 'foam-like' behavior to 'wood-like' behavior under compressive loads (Jones *et al.*, 2018; Islam *et al.*, 2018). Increased density enhances load-bearing capacity by increasing biomass per unit volume and the number of particle-to-particle contact points.

According to the results, the O-GL mycelium bio-composites demonstrated the highest compressive strength of 0,20 MPa, while the B-GL mycelium bio-composites demonstrated a compressive strength of 0,12 MPa. Statistical analysis revealed a significant difference in compressive resistance between the two bio-composites, which was attributed to the density of the prepared mycelium bio-composites.

The compressive resistance of these bio-composites is comparable to that of EPS and aligns with findings in the literature, as presented in Table 3. The compressive strength of the mycelium bio-composites, ranging from 0,123 to 0,20 MPa, meets the requirements of the Standard Specification for Rigid, Cellular Polystyrene Thermal Insulation (0,035-0,690 MPa) according to ASTM C578-18 (2018). The density of the lignocellulosic substrate influenced the mechanical properties of the final bio-composite. Substrates with higher lignin content exhibited greater density and enhanced mechanical properties, as reported in the cited literature.

## **Conclusions**

This study demonstrates that substrate type is a key parameter governing the physical and mechanical performance of mycelium-based composites. The observed differences between the B-GL and O-GL mycelium composites indicate that variations in substrate composition directly affect material density, structure, and mechanical performance.

These findings highlight the importance of substrate selection as a key design parameter for regulating the performance of mycelium-based materials. The formation of a dense, interconnected network of mycelium further highlights the role of substrate-mycelium interaction in determining mechanical behavior.

From an applied perspective, the comparable performance of these composites to expanded polystyrene (EPS) suggests that mycelium-based materials can be optimized for sustainable packaging applications, thereby contributing to the reduction of environmental impacts associated with petroleum-based materials. Accordingly, substrate selection offers a practical approach to designing sustainable, bio-based materials with tailored properties.

#### **Authorship contributions**

B.D.: Investigation, formal analysis, visualization, project administration and writing—original draft preparation. F.Y.: Conceptualization, methodology, validation, resources, and writing—review and editing.

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### Conflicts of interest

The authors declare no conflict of interest.

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