

Bio-based Strategies to Enhance the Performance of *Trametes versicolor* Mycelium-Based Leather-Like Materials

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Abstract – Mycelium-based materials are emerging as promising bio-derived alternatives to animal and synthetic leather. However, their practical implementation is limited by insufficient mechanical strength and high surface hydrophilicity, which compromise dimensional stability and durability under humid conditions. This study investigates post-processing strategies aimed at enhancing structural integrity and improving surface hydrophobicity – an essential parameter to increase water resistance and extend service life – of *Trametes versicolor* mats produced through liquid-state surface fermentation (LSSF). A preliminary assessment showed that hot pressing increased the ultimate stress of glycerol-treated sheets from 0.31 ± 0.06 to 0.45 ± 0.06 MPa, confirming its suitability as a standard consolidation step. Subsequently, sorbitol- and citric acid-treated mats were coated with seven bio-based formulations, including proteins, polysaccharides, shellac, and wax–oil blends, and evaluated through static water contact angle (WCA) measurements. The uncoated mat exhibited the highest hydrophobicity ($99.8 \pm 15.0^\circ$), while coated samples showed WCAs ranging from 65.1° (corn zein) to 95.3° (wax–oil balm). Coatings rich in hydrophobic long-chain molecules yielded the best water repellence, whereas protein- and polysaccharide-based films primarily altered surface texture without enhancing wettability. These results highlight the importance of formulation chemistry – particularly plasticizer content – in determining surface hydrophobicity, and provide a foundation for the development of scalable, fully bio-based surface finishing treatments for fungal leather-like materials.

Keywords – Biomaterials; biotechnology; sustainable materials; fungal biotechnology; fungal leather-like material.

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1. INTRODUCTION

The environmental impact of conventional leather production, together with the growing demand for bio-derived materials, has stimulated interest in mycelium-based alternatives [1], [2]. Synthetic leather does not provide a sustainable substitute, as it is typically derived from fossil-based polymers and is not biodegradable [3], [4]. In contrast, fungal mycelium-based materials have gained considerable attention – particularly from an industrial perspective – due to their ease of cultivation, rapid growth, and low carbon footprint, offering a renewable platform for the development of leather-like materials [5]–[9]. Basidiomycetes are especially suitable for material fabrication because their complex hyphal architectures enable the formation of cohesive and mechanically stable mycelial matrices [10]–[13].

The mechanical stability of mycelium-derived materials has been previously studied and quantified. Reported tensile strengths range from ~0.24 MPa for heat-pressed mycelium composites to more than 12 MPa for chemically treated and reinforced mycelium mats intended for textile applications [14]–[16].

In the present study, mats based on *Trametes versicolor* are investigated. *T. versicolor* is a white-rot fungus belonging to the family *Polyporaceae* and has gained increasing attention for the development of mycelium-based biomaterials [17]. It is widely recognised as an efficient lignin degrader, which enables growth on lignocellulosic residues and low-value agricultural substrates, thereby supporting circular bioeconomy approaches [18]. Overall, it exhibits a trimitic hyphal system composed of generative, skeletal, and binding hyphae, a structural organisation associated with increased tensile strength and stiffness of the resulting mycelial networks [19]. Recent advances in liquid-state surface fermentation (LSSF) have further enabled the controlled formation of homogeneous fungal mats suitable for downstream tailoring of mechanical and surface properties [16], [20]. Despite this progress, several challenges remain before mycelium-based materials can meet application requirements. The challenges include mechanical performance, scalability, and limited process standardization [16], [21], [22]. In addition, many fungal biomaterials exhibit an intrinsically hydrophilic surface, resulting in poor resistance to moisture and reduced dimensional stability, as reported by Amobonye *et al.* [8]. While mechanical consolidation remains a key requirement, surface properties such as wettability, tactile perception, and visual appearance are equally critical for leather-like applications. In this context, post-processing therefore plays a crucial role in refining the performance of fungal sheets. Indeed, the combined effects of plasticization, consolidation, and fully bio-based finishing treatments are still insufficiently explored. Previous studies have largely focused on chemical cross-linking, hot pressing, or coatings based on synthetic polymers. By contrast, natural coating systems – proteins, polysaccharides, waxes, and plant-derived resins – open promising routes for functionalization. Understanding how these treatments influence hydrophobicity is essential not only for improving durability and user perception but also for modulating degradability, thereby tuning the balance between performance and end-of-life behaviour. The present study investigates post-processing strategies for *Trametes versicolor* mats produced via LSSF. The paper focuses on two complementary steps:

- (i) hot pressing, evaluated for its contribution to mechanical consolidation; and
- (ii) bio-based surface coatings applied to sorbitol–citric acid treated mats to assess their effects on wettability and tactile properties.

By integrating mechanical testing with water contact angle measurements, this work provides a comprehensive assessment of how natural coatings influence the functionality of mycelium-based leather-like materials, offering guidance for the scalable and sustainable development of next-generation bio fabricated materials.

2. METHODS AND METHODOLOGY

2.1. Fungal Strain and Culture Media

A *Trametes versicolor* strain (SV1) isolated from Park Schaveys (Linkebeek, Belgium) [23] was used in all experiments. Species identity was confirmed by ITS (Internal Transcribed Spacer) sequencing followed by BLAST analysis against the NCBI database. Three main media were prepared:

- CYM (Complete Yeast Medium): 20 g/L D-glucose monohydrate, 3 g/L peptone, 2 g/L yeast extract, 0.5 g/L MgSO_4 , and 1.2 mM phosphate buffer (PB) were used. For solid cultures, 15 g/L agar was added to CYM and poured into 60 mm Petri dishes.
- ME (Malt Extract Medium): 20 g/L malt extract were dissolved in deionized water.
- GBM (Grain-Based Medium): 39 g/L finely ground wheat grains and 0.9 mL/L corn steep liquor (CSL) were mixed with deionized water.

All media were sterilized by autoclaving at 121 °C for 20 min, which corresponds to the universally recognized standard moist-heat sterilization condition used to ensure the inactivation of microorganisms and bacterial spores. CYM and ME supported precultures; GBM served as the substrate for liquid-state surface fermentation. The SV1 strain was maintained at 4 °C on CYM supplemented with agar, phosphate buffer, and antibiotics.

2.2. Inoculum Preparation and Surface Fermentation

Agar plugs ($\approx 2 \times 2$ mm) from CYM plates were transferred into 20 mL ME Falcon tubes and incubated statically at 25 °C for 7 days to form a surface mat. The mat was homogenized for 10 s and used to inoculate 180 mL fresh ME in a 500 mL flask (135 rpm, 25 °C, 6 days). The resulting suspension was homogenized for 20 s and used as inoculum for surface fermentation. Surface cultures were established in polypropylene boxes (50 mL inoculum + 450 mL GBM) or stainless-steel trays (200 mL inoculum + 1.8 L GBM). Boxes were incubated statically at 25 °C for ~ 7 days, trays for ~ 14 days, until cohesive mats formed on the liquid surface. Figure 1 schematically illustrates the workflow adopted to produce *T. versicolor*-based mycelium mats through liquid-state surface fermentation.

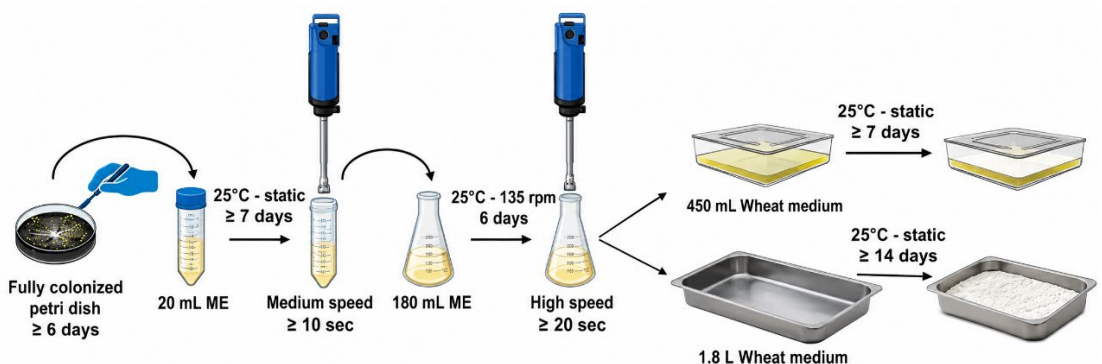


Fig. 1. Overview of inoculum preparation and liquid-state surface fermentation (LSSF) for *Trametes versicolor*-based mycelium mat production. Inoculum Steps: Mycelium from a fully colonized petri dish (≥ 6 days) is transferred to 20 mL ME medium (25 °C static, ≥ 7 days) and homogenized at medium speed (≥ 10 s). It is scaled up to 180 mL ME (25 °C, 135 rpm, 6 days) and homogenized at high speed (≥ 20 s). LSSF Scale-Up: 450 mL Wheat Medium: Incubated static at 25 °C for ≥ 7 days in a plastic container, and 1.8 L Wheat Medium: Incubated static at 25 °C for ≥ 14 days in a metal tray, producing a thick white mycelium mat.

2.3. Harvesting and Treatment

T. versicolor-based mycelium mats were manually removed, rinsed with deionized water to eliminate medium residues, and drained on stainless-steel grids for 5–10 min. After draining, as schematically illustrated in Fig. 2, mats were subjected to two different post-processing pipelines:

- For hot-pressing evaluation, mats were immersed for 2 h into a solution containing 200 g/L glycerol, dried for 48 h at room temperature under ventilated hood and then hot pressed at 100 °C for 1 min.
- For coating evaluation, sheets were first treated with a solution containing 200 g/L sorbitol and 10 g/L citric acid, dried for 48 h under ventilated hood, and subsequently coated with seven different bio-based formulations.

All coatings were applied at room temperature. Prior to hydrophobicity assessment, the samples dried for 48 h under ventilated hood and then hot-pressed at 100 °C for 1 min. The following coatings were investigated:

- Shellac: 300 g/L in ethanol + 15 g/L glycerol, applied using a cotton rag.
- Biobinder: brushed directly.
- Soy proteins: 100 g/L, 10 g/L glycerol, 10 g/L citric acid, brushed.
- Casein: 120 g/L, 15 g/L glycerol, 10 g/L citric acid, applied manually with a brush.
- Wax-oil balm: candelilla wax : coconut oil = 2:1, melted at 70 °C, applied warm with a cotton rag.
- Corn zein: 100 g/L in 900 g/L ethanol + 10 g/L glycerol, brushed while warm.
- Nanocellulose: 89 g of Cellox nanocellulose hydrogel (0.25 wt%), 10 g/L glycerol, and 10 g/L citric acid, applied as an aqueous coating.

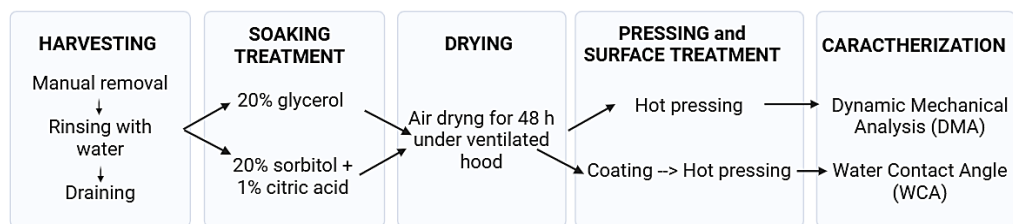


Fig. 2. Process flow diagram of post-processing treatments applied to *Trametes versicolor* mycelium sheets.

2.4. Mechanical Characterization

Tensile tests were carried out using a 5ST series electromechanical Universal Tester (Tinius Olsen, UK). Samples were laser-cut into dog-bone shapes according to the ISO 3376 standard for mechanical and physical testing of leather. The strain rate was set at 100 mm·min⁻¹. Sample dimensions are indicated in Fig. 3(a). Three technical replicates were tested for each condition. All samples were plasticized by immersion in a 200 g/L glycerol solution prior to testing, in order to reduce brittleness and minimize premature fracture during handling and tensile deformation, thereby enabling a more reliable assessment of mechanical response. For comparative evaluation of mechanical performance, hot-pressed and not-pressed samples prepared under same treatment conditions were analysed. No statistical analyses were performed.

In addition, mechanical tests were performed in tensile mode using a DMA Q800 Dynamic Mechanical Analyser manufactured by TA Instruments. For the analysis, five samples with dog-bone shape were cut from each developed mat, all plasticized with 200 g/L glycerol. The dog-

bone shaped specimens were cut via laser according to ISO 9073-3:2023, see Fig. 3(b). During the measurements, a constant loading rate of $3 \text{ N} \cdot \text{min}^{-1}$ was applied, with a 0.01 N preload and a maximum force of 18 N . Ultimate stress and strain were extracted from stress–strain curves. For the comparative evaluation of mechanical performance, tests were conducted on both hot-pressed and non-pressed samples prepared under identical treatment conditions. No statistical methods were applied.

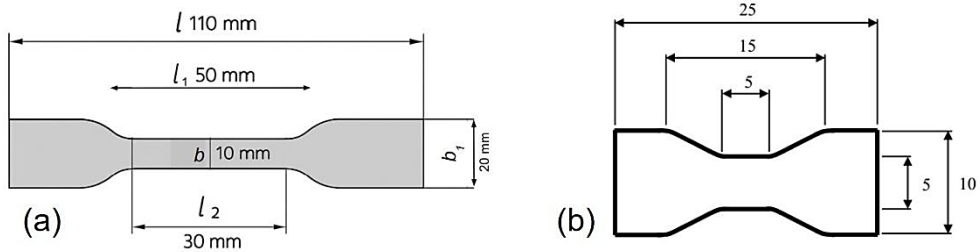


Fig. 3. Dimensions (in millimetres) of the dog-bone-shape samples of *T. Versicolor*-based mycelium mats prepared for (a) tensile tests and (b) dynamic mechanical analysis (DMA). Tensile Test Sample: Total length $l = 110 \text{ mm}$, narrow section length $l_1 = 50 \text{ mm}$, gauge length $l_2 = 30 \text{ mm}$, narrow section width $b = 10 \text{ mm}$, and end width $b_1 = 20 \text{ mm}$. Dynamic Mechanical Analysis (DMA) Sample: Total length of 25 mm , narrow centre section length of 5 mm , tapered transition section length of 15 mm , narrow section width of 5 mm , and overall width of 10 mm .

2.5. Water Contact Angle (WCA)

Hydrophobicity was quantified by static water contact angle (WCA) measurements. Indeed, the contact angle was interpreted as an indicator of wettability, ranging from 0° (fully hydrophilic) to 180° (fully hydrophobic). The wetting behaviour of a surface reflects the balance between surface tension forces at the solid–liquid–air interface. When a water droplet spreads extensively on a surface, the contact angle is low, indicating hydrophilic character. Conversely, limited spreading results in higher contact angles, corresponding to more hydrophobic behaviour. Conventionally, surfaces with WCA values below 90° are considered hydrophilic, whereas values approaching or exceeding 90° indicate hydrophobicity. A schematic representation of low and high water contact angles is shown in Fig. 4. The WCA was therefore used as a quantitative indicator of surface wettability in the present study.

Rectangular strips ($50 \times 5 \text{ mm}$) were cut randomly from each sheet. A $2 \mu\text{L}$ droplet of deionized water was deposited on the surface, and images were taken 10 s after deposition using an inverted microscope camera (16 MP). Seven droplets were deposited in different positions on each mat, so that seven measurements per sample were collected. Therefore, no statistical tests were applied. WCAs were calculated with ImageJ open-source software using the best-fit circle and ellipse models.

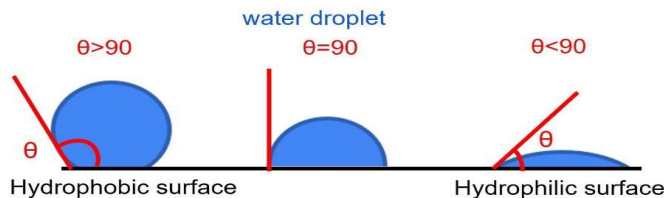


Fig. 4. Schematic representation of water contact angle (WCA) illustrating hydrophobic (high θ) surface behaviour and hydrophilic (low θ). Hydrophobic Surface (left): A rounded water droplet with a high contact angle ($\theta > 90^\circ$), Intermediate State (centre): A hemispherical water droplet with a perpendicular contact angle ($\theta = 90^\circ$); Hydrophilic Surface (right): A flattened water droplet with a low contact angle ($\theta < 90^\circ$).

3. RESULTS AND DISCUSSION

3.1. Hot Pressing Effect

A preliminary assessment examined the influence of hot pressing on the mechanical behaviour of glycerol-treated mycelium sheets. Investigations on tensile stress were performed using the universal testing machine and the tensile mode analysis conducted by DMA. As shown in Table 1, both testing configurations indicate that hot-pressing increased the ultimate stress, indicating improved compaction and structural cohesion. Hot pressing markedly enhanced the mechanical strength of the mycelium sheets, reflecting a substantial improvement in cohesion and structural integrity. These results confirm that the application of pressure promotes densification of the mycelial network, improving inter-hyphal adhesion and reducing internal porosity. Based on these findings all subsequent samples were hot pressed.

TABLE 1. EFFECT OF HOT PRESSING ON THE MECHANICAL PERFORMANCE OF *T. VERSICOLOR*-BASED MYCELIUM SHEETS

Sample	Condition	Ultimate stress – Tensile Test (MPa)	Ultimate stress – DMA (MPa)
200 g/L Glycerol	Unpressed	0.12 ± 0.04 MPa	0.31 ± 0.06 MPa
	Hot-pressed	0.42 ± 0.01 MPa	0.45 ± 0.06 MPa

Previous studies on *Formitella fraxinea* reported substantial stiffness increases after hot pressing [20]. However, the variability in species, plasticizer content, and pressing parameters across studies prevents direct benchmarking. The limitation in meaningful inter-study comparison underscores the need for standardized processing protocols.

3.2. Surface Coatings Evaluation

Sorbitol–citric acid treated mats were coated with seven fully bio-based formulations, including proteins, polysaccharides, shellac, and wax–oil blends, and evaluated through static water contact angle (WCA) measurements. Currently, to the best of our knowledge, only a limited number of studies has investigated direct coatings applied to mycelium sheets. The studies are mainly reporting hydrophobic corn zein layers [24]–[26] and wax–oil blends [27], [28] as promising approaches. Other bio-based systems considered in this work, e.g., shellac, nanocellulose- or soy-based formulations, [29]–[31] were selected based on their known film-forming or barrier properties in different contexts. A prior validation specifically on mycelium substrates is absent. In the current study, the bio-based coatings were applied to enhance the surface hydrophobicity and aesthetic properties of the *Trametes versicolor*-based mycelium mats.

All coated mats retained good flexibility and flatness, although differences in odour intensity and surface tackiness were observed. Table 2 lists the results for 7 samples and the used control as reference. The control corresponded to sorbitol and citric acid-treated and hot-pressed mats that did not receive an additional surface coating. Biobinder (Sample 1), nanocellulose (Sample 2), and wax–oil balm (Sample 4) exhibited mild odour, whereas soy (Sample 5), casein (Sample 6), and zein (Sample 7) coatings emitted a stronger smell linked to organic residues and slow moisture release. The biobinder-coated sample (Sample 1) remained distinctly sticky even after hot pressing, making it unsuitable for practical use; therefore, it was excluded from WCA analysis. Water contact angles for the remaining coatings are reported in Table 2.

TABLE 2. SUMMARY OF COATING FORMULATIONS, APPLICATION METHODS, QUALITATIVE OBSERVATIONS, AND WATER CONTACT ANGLE (WCA) RESULTS FOR *T. VERSICOLOR*-BASED MYCELIUM MATS. THE CONTROL CORRESPONDED TO SORBITOL AND CITRIC ACID-TREATED AND HOT-PRESSED MATS THAT DID NOT RECEIVE AN ADDITIONAL SURFACE COATING

Sample	Coating formulation	Application method	Observation	WCA, °
Sample 1	Pure biobinder	Brushed	Homogeneous Low gloss No colour changes Sticky even after hot pressing	—
Sample 2	89 g Cellulose Cellox (0.25%) + 10 g/L glycerol + 10 g/L citric acid	Brushed	Homogeneous Matte No colour changes Velvety surface	85.2 ± 8.4
Sample 3	Shellac 300 g/L (in ethanol) + 15 g/L glycerol	Rag-applied	Heterogeneous Glossy Orangish tone Minor surface cracking	73.1 ± 7.8
Sample 4	Wax-oil balm (candelilla wax + coconut oil 2:1)	Warm rag-applied	Homogeneous Low gloss No colour changes Slightly greasy	95.3 ± 9.9
Sample 5	Soy proteins 100 g/L + 10 g/L glycerol + 10 g/L citric acid	Brushed	Homogeneous Low gloss Slightly yellowing Slightly stiff surface	74.5 ± 7.3
Sample 6	Casein 120 g/L + 15 g/L glycerol + 10 g/L citric acid	Brushed	Homogeneous Low gloss Yellowing Slightly stiff surface.	80.9 ± 10.3
Sample 7	Corn zein 100 g/L + 900 mL/L ethanol + 10 g/L glycerol	Brushed	Heterogeneous Semi-gloss Yellowing Slightly rigid surface	65.1 ± 9.6
Control	—	—	Homogeneous Low gloss No colour changes Smooth	99.8 ± 15.0

It is important to note that several formulations (shellac, soy, casein, nanocellulose) contained glycerol, a highly hydrophilic plasticizer known to increase water affinity in biopolymer films [32], which may have contributed to reduced WCAs. Therefore, the results indicate that under the specific compositions and processing conditions used, coatings did not surpass the hydrophobicity of the untreated hot-pressed control mats. Representative images of all coated samples, before and after hot pressing, are shown in Fig. 5.



Fig. 5. Images of the *T. versicolor*-based mycelium mats before (left) and after (right) hot pressing at 100 °C for 1 minute. All mats received additional surface coating except for the control mats.

The control sample, i.e., the mat without additional surface coating, exhibited the highest WCA ($100 \pm 15^\circ$). Among coated samples, the wax–oil balm (Sample 4) showed the best water repellence ($95 \pm 10^\circ$), consistent with the hydrophobicity of long-chain fatty compounds. Nanocellulose (Sample 2) achieved relatively high values ($85 \pm 8^\circ$) and produced a smooth matte surface with a soft “velvet-like” feel, although the hydrophilic nature of cellulose inherently limited its performance. Intermediate WCAs were obtained for casein ($81 \pm 10^\circ$) and soy protein ($74 \pm 7^\circ$) coatings, which formed homogeneous but opaque, slightly yellow films. Shellac

(Sample 3) produced a glossy but brittle surface with microcracks visible after drying, resulting in a WCA of $73 \pm 8^\circ$. The corn zein coating (Sample 7) yielded the lowest WCA ($65 \pm 10^\circ$) and showed poor homogeneity.

Overall, coatings containing hydrophobic long-chain molecules (waxes and oils) were the most effective in maintaining or enhancing water hydrophobicity. However, it should be taken into account that static WCA measurements only provide information on the initial wetting behaviour of the surface, but do not fully describe water resistance under real-life use conditions. Short-term hydrophobicity is only one relevant parameter, but water uptake over longer exposure times, swelling, coating adhesion and abrasion resistance under humid conditions are also important. Thus, an increased WCA does not necessarily imply long-term moisture resistance. In contrast to waxes and oils, protein- and polysaccharide-based coatings mainly modified surface texture and appearance rather than affecting wettability.

4. CONCLUSIONS

In this study post-processing treatments were investigated for improving the performance of mycelium-based mats produced via liquid-state surface fermentation. Seven bio-based coatings were evaluated in terms of ability to modify surface hydrophobicity and appearance. The study with tensile tests was conducted to assess the effect of hot-pressing on the physical and mechanical properties of mycelium-based sheets. In the tensile test, the ultimate stress increased from 0.12 ± 0.04 MPa in not-pressed samples to 0.42 ± 0.01 MPa after hot pressing, while in DMA tensile mode the ultimate stress increased from 0.31 ± 0.06 MPa to 0.45 ± 0.06 MPa. Hot pressing increased the tensile strength of glycerol-treated mats, providing the role as an effective consolidation method for enhancing structural cohesion.

Among the tested formulations, wax-oil balm provided the highest WCA ($95 \pm 10^\circ$) and produced a flexible, mildly greasy surface consistent with its hydrophobic chemistry. Nanocellulose, casein, and soy protein coatings generated homogeneous films with distinct tactile and optical properties but did not improve hydrophobicity. Shellac and corn zein yielded glossy or semi-transparent layers yet exhibited limited or reduced water repellence, partly attributable to microcracking and the presence of glycerol in the formulations.

Overall, none of the coatings surpassed the hydrophobicity of the uncoated control, highlighting the strong influence of hydrophilic additives, particularly glycerol, on surface wettability. The results highlight the need to optimize chemistry formulation, particularly plasticizer content and film-forming behaviour, to achieve effective water-repellent bio-based coatings.

The present study entails an initial comparative screening of bio-based post-processing strategies rather than being a fully optimized coating study. One can mention two important drawbacks: first, the current number of published studies specifically addressing coatings on pure mycelium sheets remains limited, which restricts direct benchmarking. Second, standardized protocols for production, plasticization, coating, hot pressing, mechanical characterization and WCA measurements of pure mycelium materials are currently not yet available. These limitations complicate comparison and benchmarking. In addition, the used coating formulations differed not only in chemical composition but also in application method and plasticizer content. Nevertheless, by identifying methodological constraints, our study provides a useful starting point for more systematic optimization of bio-based coating strategies. Thus, this study offers a contribution to the very promising research to develop and design fully bio-based and fully biodegradable fungal-based leather-like materials.

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