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Mycelium-Based Leather: A Review on Post-Processing Treatments and Material Enhancements

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***Abstract* – Textile and fashion industry is one of the most polluting industrial sectors in the world, responsible for high greenhouse gas emissions and water contamination. Leather tanning is a non-eco-friendly process, which consumes large amounts of water and energy while potentially releasing heavy metals and toxic compounds into the environment. Although synthetic leather offers simplified production processes, it is derived from fossil fuels and is non-biodegradable, raising further environmental concerns. Filamentous fungi, with their ability to form leather-like biomaterials through controlled fermentation, represent a promising and sustainable alternative. Critical parameters like fungi species selection, nutrient composition, growth conditions and treatment techniques are analysed. Distinct types of post-processing treatments that can enhance mechanical performance, durability, and water resistance are discussed highlighting the potential of mycelium-based materials as a sustainable alternative to conventional leather.**

***Keywords* – Biomaterials; filamentous fungi; fungal biotechnology; leather; leather-like materials; sustainable fashion; tanning; textile.**

1. INTRODUCTION

The environmental impact of the traditional leather industry has long been a global concern. As one of the oldest materials used by human civilization, animal-derived leather is highly valued for its durability, versatility, excellent visual and tactile properties. According to Fortune Business Insights [1], the global leather goods market size was valued at USD 498.57 billion in 2024 and it is projected to grow from USD 531.07 billion in 2025 to USD 855.36 billion by 2032, exhibiting a CAGR of 7.05 % during the forecast period. However, the leather production processes pose significant risks to both human health and environment. Recent trends, however, have indicated an increasing consumer awareness, especially in the Global North, regarding the leather industry and the sustainability of its processing [2]. The conventional leather tanning process generates a variety of pollutants, leading to severe environmental consequences. Wastewater from leather production contains high levels of chemical oxygen demand, biological oxygen consumption, and total suspended solids [3]. Indeed, the tanning step is notorious as the most environmental degrading step due to its huge

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water consumption and its potential release of hazardous chemicals including chromium salts, chlorides, sulfides, tannins, oils, resins, and detergents, into the surrounding ecosystem [4], [5]. This step stabilizes the collagen fibers by inducing cross-linking bonds, which keep the fibers separated and reinforce the overall structure. Further effects of cross-linking are an increase in hydrothermal stability, higher resistance of collagen to enzymatic degradation and increased tensile strength. Nowadays, about 90 % of the leather is tanned with trivalent chromium, an environmentally hazardous cross-linker that has led the leather industry to reintroduce tannins of vegetable origin [6]. Chromium used in leather manufacturing can be oxidized from its trivalent form (Cr^{3+}) to the hexavalent state (Cr^{6+}), a highly hazardous state. Cr^{6+} is known to bioaccumulate and poses severe risks to human health due to its high membrane penetration and strong oxidizing ability, making it highly toxic, mutagenic, and carcinogenic [7]. It is ranked 18th on the Priority List of Hazardous Substances [8]. To address these issues associated with the traditional leather industry, it is imperative to develop leather alternatives that replicate the appearance, texture, and durability of animal-derived leather. The development of synthetic alternatives, including polyvinyl chloride, polyurethane, and ethylene vinyl acetate [3] has been primarily driven by their low production costs and few by-products. Although these materials offer advantages in terms of simplified production, they are primarily derived from fossil fuels and are non-biodegradable, leading to concerns over energy consumption, climate change, and long-term ecological effects. Consequently, research has shifted toward developing more sustainable and bio-based alternatives to synthetic leather, including materials from bacterial cellulose, banana trunk fibers, cactus leaves, mango fibers, pineapple leaves, lab-grown collagen, and fungal mycelium. Among these alternatives, mycelium-based leather has gained considerable attention, especially from an industrial point of view, due to its ease of cultivation, rapid growth cycle, and lower carbon footprint [4], [5].

2. MYCELIUM LEATHER-LIKE MATERIALS

Filamentous fungi exhibit a natural growth pattern which generates an extending amount of microscopically interconnected tubular cells eventually adding up to form the mycelium network, their vegetative phase. Fungal mycelium is made up of chitin, glucans and proteins and can be used to create some biomaterials that, nowadays, have become a focus of various start-ups and established companies in the world because of their sustainability, low combustibility potential, low production cost, fast growth rate as well as their relatively low carbon footprint and carbon turnover [2]. According to Elsacker *et al.* [9], ca. 70 organisms were identified across granted and non-granted patents. *Ganoderma* spp. and *Trametes* spp. are the most listed genera in patent descriptions, followed by *Fomes*, *Fusarium*, *Pleurotus*, and *Schizophyllum*. Except for *Fusarium*, which is an *Ascomycete*, all the above-mentioned species are members of the Basidiomycetes. The *Basidiomycota* order of *Polyporales*, known for their wood-decay properties, are particularly suited for biomaterial applications due to their hyphal structure and tuneable cell-wall composition, which influence their mechanical properties and adaptability [10]. Their fruiting bodies vary from soft to tough because of their hyphal structures and cell-wall chemistry. The cell-wall polymer architecture and chemical composition (glucan and chitin) greatly influence external ecological factors. In addition, the physio-mechanical properties of mycelia are affected by the substrate type, incubation conditions, and fabrication process. However, selecting suitable strains and conditions for mycelium-based composites and leather production is complex. A monokaryotic culture is typically the preferred option; however, hybridization and mutation may improve the strength, flexibility, and durability of the mycelium mat. Some of the eligibility criteria regarding the selection of the strains include mycelium density, growth rate, ease of cultivation, cost of the growth substrate, pathogenicity profile, and the structure of the mycelium. The cell wall composition of the fungal strain is also important as the compositional ratio of structural polymers (such as chitin/protein content) has a significant effect on the post-processing efficiency through the amount of available chemical crosslinking sites and consequently material properties. In the process of utilizing living cells as microbial factories, the optimization of key parameters is important to achieve a competitive economy of scale. The production method also significantly impacts the quality of the mycelium mat. Mycelium mat is typically produced using three methods, namely, solid-state surface fermentation (SSSF), liquid-state surface fermentation (LSSF), and submerged fermentation (SubF). SSSF uses solid substrates where the mycelium grows upwards from the top surface, to consume oxygen. Once the mycelium mat has formed, it is subjected to hot pressing to prevent the further growth of the fungi and chemical treatments to improve its characteristics. This type of fermentation provides benefits such as the use of waste materials, less capital cost, and elevated production but it faces difficulties in maintaining consistent texture and yield. Additionally, it might be also vulnerable to contamination throughout the scaling-up process. Therefore, more studies are required to evaluate the productivity and contamination rates of SSSF in mycelium mat production under different conditions [11]. LSSF uses liquid medium or waste lignocellulosic substrates, under static conditions, allowing mycelium to develop on the surface. After fungal mycelium fermentation and growing in the liquid medium, the fibers are then separated, and undergo post-processing to achieve a flexible material similar to bovine leather. Additionally, the material properties of mycelium biomass can be enhanced by incorporating fibrous materials, such as linen or cotton, onto the surface, allowing the mat to integrate with the layer. However, a key challenge in LSSF is the limited availability of dissolved oxygen, which can hinder biomass proliferation [2]. SubF involves cultivating a fungal species in a stirred submerged liquid suspension. The fungal biomass is typically produced as slurry or pellets, which are blended, homogenized and plasticized to create flat, leather-like sheets. Although SubF allows precise control and high biomass yields, it is more expensive and labour-intensive than static fermentation especially because an active stirring is required [12], [13]. Fig. 1 reports the key steps affecting the production of mycelium leather-like mats, which can be schematically summarizes in the following four processes: (i) Selection of the fungal strain: e.g. *Ganoderma*, *Trametes*, *Fomes*, *Fusarium*, *Pleurotus*, *Schizophyllum* etc. based on growth characteristics, mechanical properties and texture; (ii) fermentation: cultivation of the mycelium under controlled conditions; (iii) harvesting: collection of the mycelium sheets; (iv) post-processing treatments: several treatments to enhance mechanical properties, durability, and aesthetics, including drying plasticization, cross-linking, oxidation resistance, coating treatments and reinforcement.

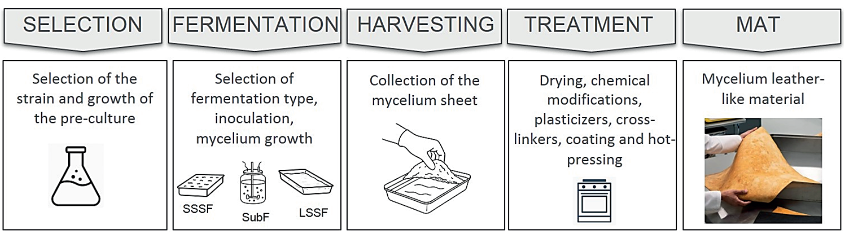

Fig. 1. Key steps affecting the production of mycelium leather-like mats.

Williams *et al.* [14], in a recent study conducted for the company MycoWorks, have demonstrated that mycelium-based leather substitute exhibited a significantly lower carbon footprint of 2.76 kg CO_2 eq/m^2, which is 94 % lower than that of cow leather. It is to note that, despite its environmental benefits, pure mycelium composites currently face limitations in terms of high variabilities, mechanical properties, scalability, and the absence of a standardized production process, as discussed in the following section.

3. CHALLENGES WITH MYCELIUM MAT PRODUCTION

Mycelium-based leather faces several challenges related to the mechanical properties, scalability and standardization. Pure mycelium–based composites do not currently meet the desired criteria in terms of physicochemical and sensory properties such as tensile strength, handling characteristics and uniform thickness. In a recent study, Raman *et al.* [10] have compared the properties of the mycelium-based leather with respect to the animal (bovine) leather. They tested different mycelium strains: *Fomitella fraxinea*, *F. rosea. Ganoderma lucidum*, *Ganoderma applanatum*, *Elfvingia applanata*, *Bjerkandera adusta*, *Microporus affinis*, *Trametes versicolor*, *Trametes suaveolens*, *T. hirsuta*, *Fomitopsis pinicola*, *Wolfiporia extensa*, *Postia balsamea*; the results are summarized in Table 1.

TABLE 1. PHYSICAL PROPERTIES OF MYCELIUM-BASED LEATHER AND BOVINE LEATHER [3], [10]

Sample	Elongation, %	Strain	Tensile Strength, MPa	Young's Modulus, MPa
Mycelium-Based leather	4.59–58.86	1.03–1.49	1.40–8.49	1.37–8.14
Animal (bovine) leather	<40	6–16	39.5	1–13

Additionally, Ambonye *et al.* [2] have noticed an undesirably hydrophilic propriety. While some fungal species demonstrate promising strength, mycelium-based materials generally lack dimensional stability, flexibility, and resistance to biological and chemical degradation. Furthermore, efforts to achieve the desired properties often involve chemical processing, which can compromise the environmental sustainability of the material. Alternative solutions include genetic modification, process optimization, and the incorporation of biodegradable additives such as cellulose, chitosan, or lignocellulosic biomass. Hybridization with synthetic materials like polyurethane has also been explored, though it raises concerns about biodegradability. Scalability remains a major obstacle, as mycelium cultivation is a complex biological process influenced by long production cycles, contamination risks, and multi-step manufacturing procedures. The lack of consistency and reproducibility of a specific material profile, therefore, prevents the scaling up of mycelium in both size and quantity to reach an industry-standard level of product certification [15]. The testing and standardization process for these new materials is further burdened by the vast quantity of possible treatments and supporting materials which can be used in combination with mycelium biomass to achieve a finished leather-like product. Consequently, different standard tests are needed to accurately characterize the materials, considering that mycelium is almost always combined with different elements to attain its functional role as leather-like material. Significant efforts are being made to overcome these challenges, as mycelium-based leather is considered one of the most promising and viable alternatives to conventional leather. To improve the mechanical properties, crosslinking the mycelial fibers is expected to enhance strength, toughness, and durability [3].

4. Post-Processing Treatments

Mycelium-based materials become stiff and brittle when fully dried. To avoid such inconvenient conditions, a series of physical and chemical treatments need to be applied to achieve the desired aesthetics, shape, texture and mechanical properties required for end use. Among these treatments, green chemistry cross-linking of mycelial fibers plays a crucial role in enhancing stiffness, strength, and durability. The different combinations and variations of treatments currently in use primarily aim to improve flexibility, as well as increase the durability and absorbent properties. However, the treatment processes are still undergoing intensive research, and there is currently no universally applicable procedure. Additionally, the specific processing methods used by various companies in the development of fungal leather substitutes remain proprietary trade secrets, making an authoritative comparison difficult.

4.1. Green Chemical Modifications

4.1.1. Deacetylation: from chitin to chitosan

The mycelial cell walls of fungi consist of polysaccharides (e.g., chitin and glucan) and glycoproteins. Polysaccharides serve as structural components, while glycoproteins (such as mannoproteins, galactoproteins, xylomannoproteins, and glucuronoproteins) function as interstitial components of the fungal cell walls. The reaction of deacetylation allows to transform chitin into chitosan, enhancing the material's flexibility and mechanical properties. Chitin, the main component of the fungi cell wall, is a linear biopolymer formed by N-acetyl-D-glucosamine units linked by glycosidic β (1,4) bonds. Chitin is the second most abundant polysaccharide in nature after cellulose, and it can also be found in the exoskeletons of crustaceans and mollusks and in insect cuticles [16]. Chitin contains hydroxyl and acetamide groups, see dotted cycles in Fig. 2.

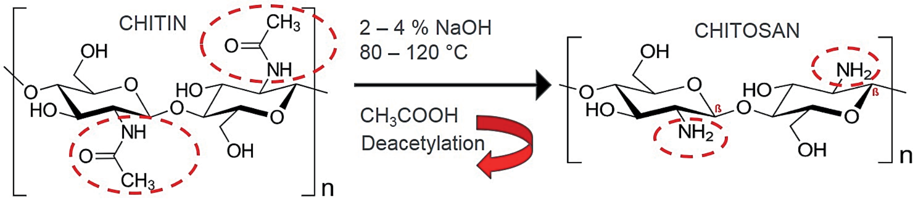

Fig. 2. Deacetylation reaction process to obtain chitosan from chitin of fungi cell walls.

The acetamide groups are deacetylated to amine groups by a deacetylation process (shown by dotted cycles in Fig. 2): chitin can be converted into chitosan, which possesses free amine groups. Chitosan is soluble in aqueous acid such as acetic acid and lactic acid, where its solubility depends on the deacetylation degree and molecular weight; a higher degree of deacetylation and lower molecular weight translate into increased solubility. Chitosan has a relatively stable chemical structure and exhibits polycationic, non-toxic, biodegradable, and innocuous properties. It is also biocompatible with various organs, tissues, and cells. Chitosan molecules are physically and biologically active, allowing for chemical or enzymatic modifications tailored to specific applications [16]. In the context of mycelium-based leather, the free amine groups of chitosan can participate in cross-linking reactions, leading to improved mechanical strength and durability. Additionally, deacetylation has been shown to increase the thermal stability, fire resistance and antimicrobial proprieties of mycelium materials, providing a distinct advantage over their synthetic counterparts. Traditionally the deacetylation of the chitin is performed by an alkaline treatment (such as 2–4 % NaOH) at high temperatures (80–120 °C) for several hours, as reported in Fig. 2.

4.1.2. Oxidation: from hydroxyl groups to carboxyl groups.

Oxidation is a key chemical modification which increases the reactivity of chitin, the major cell wall component of mycelium-based leather, supporting its potential as a sustainable alternative to conventional leather. Indeed, in the context of mycelium-based leather, oxidation introduces carboxyl and carbonyl functional groups into the polysaccharide structure of the fungal cell wall. These groups improve hydrophilicity and reactivity, facilitating better interactions with cross-linking agents and plasticizers. Therefore, these chemical modifications can strengthen the material's network, leading to improved mechanical properties such as increased tensile strength and flexibility. An example of efficient oxidation of chitin is the catalytic reaction using TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical), NaClO, and NaBr in water at pH 10–11. The water-soluble polyuronic acid is produced, which has a chemical form like hyaluronic acid [17], as seen in Fig. 3.

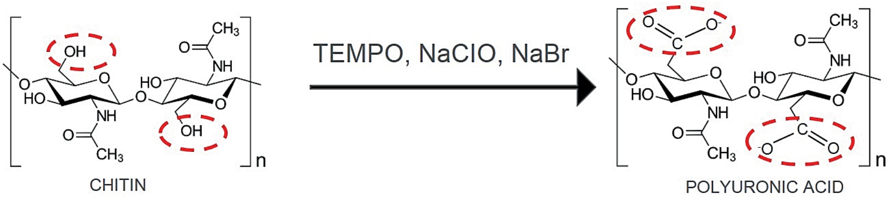

Fig. 3. Catalytic oxidation reaction process to obtain polyuronic acid from chitin of fungi cell walls.

4.2. Chemical Cross-linking

4.2.1. Chromium Cross-Linking

Mineral tanning with metallic ions is widely used in traditional leather production, with over 85 % of leather made through trivalent chromium tanning. Mycelial cell walls are rich in polysaccharides and proteins, bearing functional groups with lone pairs of electrons: COO–, O–H, C=O, C–O, and other groups. These groups can coordinate with metal cations through chelation or ion-exchange mechanisms, contributing to the metal-binding capacity of the mycelial matrix. Consequently, it is promising to promote the cross-linking between mycelial fibers and metal ions in mycelium-based leather substitute production to improve the strength and stability of the leather substitutes. Li *et al.* [3] have reached an increased tensile strength of 8.4 MPa and an elongation at break of 32.7 %, starting with a mycelium film with 4.5 MPa of tensile strength and 20 % of elongation at break, demonstrating superior mechanical properties compared to untreated mycelium. Additionally, the thermal stability of Cr^{3+}-cross-linked mycelium was significantly improved, ensuring stability even at high temperatures. However, chromium cross-linking raises environmental concerns due to the toxicity of chrome and its potential impact on sustainability. For that reason, researchers have investigated alternative cross-linking methods: the use of zinc is considered one of the best options.

4.2.2. Zinc Cross-Linking.

Zn^{2+} is a nontoxic metallic ion that can coordinate with amino and carbonyl groups in polysaccharides, forming complexes with high antioxidant activity. Zinc cross-linking enhances the mechanical properties, thermal stability, and antioxidant activity of mycelium-based leather substitutes, leading to a more compact mycelial network with increased tensile strength (from 4.5 MPa to 7.0 MPa) and elongation at break (from 20.1 % to 59.4 %). Additionally, Zn^{2+} cross-linking improves organoleptic properties and ensures thermal stability up to 230 °C, making it a viable method for enhancing mycelium-based leather durability [3].

4.3. Organic and Synthetic Cross-Linker

4.3.1. Gluteraldehyde

Glutaraldehyde (GA) is widely recognized as an effective cross-linking agent due to its ability to form covalent bonds with amino and hydroxyl functional groups in biological polymers. An example is the common use of GA as a crosslinker agent for collagen-based biomaterials [18]. GA is a dialdehyde that reacts with (hydroxy)lysine residues in collagen, forming a Schiff base. Another valid organic cross-linking agent is N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), which is a water-soluble carbodiimide that forms amide linkages between carboxyl and amino groups in proteins such as collagen. In the context of mycelium-based leather substitutes, GA can form covalent bonds with amino acids in proteins and hydroxyl residues in polysaccharides, demonstrating a significant potential for enhancing mechanical performance, enzymatic stability, and water resistance. In a recent study, d'Errico *et al.* [6] attempted treating mycelium sheets with varying concentrations of EDC and GA. As reported in Table 2, the best strength was obtained by treating with 6 % glycerol + 0.1 % GA, producing an ultimate tensile strength of 11.1 MPa and an elongation at break of 14.6 % in mats of *Schizophyllum commune*. This represents a substantial improvement over the untreated mycelium sheets, which originally had values of 7.8 MPa and 15.2 %, respectively. While the tensile strength increased significantly, the slight reduction in elongation at break suggests an enhancement in structural rigidity, making the material more resilient to stress while maintaining a degree of flexibility. Cross-linking also increased enzymatic stability and reduced mycelial water absorption but did not result in increased thermal stability. These results provide foundations to produce mycelium-based leather composites featuring a three-dimensional mesh structure, thereby significantly enhancing the sustainable utilization of low-carbon biomaterials. However, despite the advantages of GA treatment, the limited thermal stability, and the fact that it is considered hazardous for wildlife and human health, suggest that alternative treatments may be required to optimize the sustainable production.

TABLE 2. EFFECT OF TANNING ON THE MECHANICAL PROPERTIES OF MYCELIUM-BASED LEATHER MATS GROWN WITH *SCHIZOPHYLLUM COMMUNE* [3], [6]

Sample	**Ultimate tensile strength, MPa**	**Elongation at break, %**
Mycelium Mat Untreated	5.0	1.5
Mycelium Mat, 6 % Glycerol	7.8	15.2
Mycelium Mat, 6 % Glycerol + 0.1 % GA	11.1	14.6

4.3.2. Organic acids

Chemical cross-linking can be accomplished using organic acids such as citric acid, dicarboxylic acid and polycarboxylic acid. Citric acid is hypothesized to facilitate cross-linking between neighboring hyphae by reacting with the hydroxyl groups in glucan present in the cell wall [9]. In the study by Crawford *et al.* [19], citric acid was used as a crosslinking agent at different concentrations. While it influenced the color and flexibility of the samples, higher concentrations led to increased rigidity, warping, and greater susceptibility to breakage. However, citric acid concentration was not a significant factor in tensile strength. In contrast, glycerol concentration had a significant effect when applied after a citric acid bath. No significant interaction was observed between citric acid and glycerol concentrations.

Tannic acid is widely used as effective cross-linker. The reaction mechanism involves hydrogen bonds between chitosan and tannic acid and then interactions between phenolic hydroxyl groups and amino groups. In a recent study by Song *et al.* [20], different concentrations of tannic acid (3 %, 4 %, 5 %) were used to cross-link a mycelium film of *Ganoderma*. The treatment was performed at 60 °C for 12 h and the membrane was subsequently plasticized with 20 % glycerol and dried. Tensile tests show that the tensile strength of cross-linked mycelium film was significantly increased, as well as the elongation at break and the hydrophobicity.

Raman *et al.* [10] highlighted that treating mycelium-based leather with tannic acid at concentrations of 5 % and 10 % resulted in a desirable dark reddish-yellow coloration. The mycelium mat, plasticized also with 15 % glycerol and subjected to hot pressing (60 °C), was also analyzed for its mechanical properties, as summarized in Table 3 [8], [21]. These results suggest that tannic acid cross-linking compromises the flexibility and strength of the material, making it stiffer but potentially more resistant to deformation.

TABLE 3. EFFECT OF HOTPRESSING AND DIFFERENT PLASTICIZERS ON THE MECHANICAL PROPERTIES OF MYCELIUM-BASED LEATHER. MYCELIUM STRAIN: *FORMITELLA FRAXINEA* [8], [21]

Sample	Elongation rate, %	Tensile Strength, MPa	Strain	Young's Modulus, MPa
Untreated without hotpressing	4.59 ± 1.29	1.40 ± 0.22	1.03 ± 0.01	1.37 ± 0.20
Untreated, hotpressed at 60 °C	25.41 ± 3.29	2.65 ± 0.26	1.13 ± 0.02	2.48 ± 0.20
15% Glycerol, hotpressed at 60 °C	58.86 ± 5.19	4.92 ± 1.05	1.29 ± 0.03	3.79 ± 0.74
15% Glycerol + 5% Tannic Acid, hotpressed at 60°C	22.64 ± 1.15	3.13 ± 0.62	1.11 ± 0.01	2.81 ± 0.54
15 % EG, hotpressed at 60 °C	33.56 ± 5.96	5.09 ± 1.11	1.17 ± 0.03	4.35 ± 0.90
20 % PEG, hotpressed at 60 °C	18.88 ± 6.33	5.06 ± 0.78	1.17 ± 0.03	4.62 ± 0.67

4.3.3. Genipin

From the various natural cross-linker that were studied to improve sustainability of the process, genipin seems to be very promising. Genipin is a small-molecule metabolite of geniposide, found in the gardenia fruit as a chemical defence against herbivores and pathogens. It acts as a connector between two chitosan fibres, enhancing the overall strength of the material [22].

Commercially available genipin powder is typically dissolved in a solution of acetic acid and then applied to the mycelial mat, which is then incubated at 25 °C for several hours and subsequently rinsed with water. This cross-linking strategy enhances the hydrophobicity, tensile strength, and elastic modulus of the material. However, as incubation time increases, the resulting material becomes stiffer and less ductile, as observed by Elsacker *et al*. [9]. In the study by Song *et al*. [20], genipin was used at different concentrations (0.5 %, 1 %, 1.5 %) to cross-link mycelium-films. It was found that increasing the genipin concentration enhanced both the tensile strength and elongation at break, with peak values of 6.22 MPa and 18.92 %, respectively, at 1.5 % genipin. Furthermore, the higher genipin concentration led to an increased contact angle, reflecting a smoother, more uniform surface morphology and greater water resistance. According to the same study, genipin cross links with chitosan primarily through the formation of strong covalent bonds between its reactive functional groups—particularly aldehyde and ester moieties—and the primary amino groups present in chitosan. This reaction leads to the formation of a stable, three-dimensional polymer network, increasing the crosslinking density within the material [21], [22].

However, as noted by Elsacker *et al*. [9], a side reaction involving genepin can cause the material to turn blue, which may be undesirable for some applications. Therefore, optimizing the treatment protocol is essential to achieving the ideal balance between mechanical performances, colour stability, and sustainability.

4.4. Enzymatic Cross-Linking

Enzymes represent another simple and natural approach for inducing cross-linking [23]. Enzymes belonging to the polyphenol oxidase class, such as tyrosinase, laccase and peroxidase, are useful for this purpose. For example, tyrosinase converts a phenolic substrate into a more reactive o-quinone compound, which serves as the cross-linker between chitosan fibres; so, a phenolic substrate is needed for the reaction. A favorable aspect of the tyrosinase-driven reaction is that tyrosinase accepts many different phenolic substrates. While the tyrosinase cross-linking reaction is not fully understood, it appears that these reactions can occur in acidic, neutral, or alkaline conditions. Additionally, it is a low-energy and easy process. The main challenge with tyrosinase-driven enzymatic cross-linking is the lack of data on how it impacts the material's mechanical properties. As explained by Deeg *et al*. [22], this method works for cross-linking chitosan, but it is still unclear whether it will provide the strength and flexibility needed for the final product. Since tyrosinase also triggers 'browning' reactions in natural processes (like food browning), more research is needed to understand how this can impact the colour of leather-like materials.

4.5. Plasticizing Treatments.

Mycelium-based materials tend to become stiff and brittle upon drying, necessitating the use of plasticizer to maintain flexibility. Plasticizing agents reduce brittleness of films from bio-derived polysaccharides. Generally, plasticizers are low molecular weight non-volatile compounds that are incorporated in a material to increase flexibility [24]. Various classes of plasticizer can be employed, including polyols like glucans, chitin and chitosan biopolymers but also sugar alcohols, ester plasticizers and vegetable oils. Glycerol is the most used plasticizer [9].

4.5.1. Glycerol

Glycerol is a by-product of the bio-diesel production. It is a small hygroscopic polyol, which notably increases flexibility and elasticity of mycelium materials and reduces ultimate tensile strength. This compound forms hydrogen bonds with cell wall polymers, increasing the space between the cell wall components. The mechanical properties of mycelium-based materials (*Schizophyllum commune*) were significantly influenced by glycerol treatment: the increasing of the glycerol concentration was related to a decrease of Young's Modulus and ultimate tensile strength, indicating greater flexibility [19], [24]. Additionally, it was observed that glycerol treatment reduced surface roughness, thus possibly lowering surface hydrophobicity. In the patent "Methods of Generating Mycelium Materials with Improved Properties", Wang *et al*. [25] confirmed that adding glycerol helps to enhance flexibility, pliability and reduce brittleness. However, the inclusion of glycerol can influence the biodegradation rate of the materials. Its hydrophilic nature makes the materials more susceptible to microbial growth, potentially accelerating degradation under certain environmental conditions [25]. This aspect is crucial when considering the material's lifespan and environmental impact.

4.5.2. Sorbitol

Sorbitol, another polyol, functions similarly to glycerol, allowing to improve material flexibility. It has been widely used in many biopolymer-based materials to enhance elasticity and moisture retention. According to Utami Hatmi *et al*., [26] sorbitol is a reduced glucose formed by the oxidation of glucose, has a crystal form and is soluble and stable in water. When in composite, sorbitol decreases the water vapor permeability and increases the tensile strength, producing films more resistant than glycerol. Sorbitol was used to enhance mechanical properties of the cultivated mycelium material such as elasticity and flexibility. This plasticizer reduces brittleness, making the material more suitable for applications requiring softness and adaptability without compromising on barrier properties [25].

4.5.3. Polyethylene Glycol and Ethylene Glycol

Ethylene glycole, EG, and polyethylene glycole, PEG, were evaluated as plasticizer. They effectively enhance flexibility in many biopolymeric materials, including fungal-based composites. Main reason is the modification of the hydrogen bonding within the polymer matrix. Studies suggest that PEG-treated mycelium materials exhibit improved flexibility without significantly compromising mechanical integrity [10]. The highest Young's Modulus, in fact, were reached with 20 % of PEG (see Table 3). Also, in the patent "Method for Producing Fungal Mats and Materials Made Therefrom", Montalti *et al*. explained that the use of PEG and PE like plasticizers help to prevent brittleness while maintaining flexibility [27].

4.5.4. Oils

In the leather industry, fat liquors affect the leather matrix by reducing interfibrillar frictional forces, improving tensile strength, introducing softness, and protecting the leather against cracking. As explained in the patent "Nanoemulsion for internal humectation of mycelium-based textiles" by Naranjo-Briceño *et al*. [28], environmental-friendly alternatives were explored: vegetable-based oils as fat liquoring agents have been suggested such as sulfated castor oil, beeswax, coconut oil, linseed oil, oleic acid, sulfated fish oil, sulfated canola oil, soybean oil, palm oil, fatty acids. These offer environmentally friendly and cost-effective alternatives to petrochemical-based treatments. Additionally, they can provide hydrophobicity, improving water resistance while maintaining flexibility. However, their impact on mechanical properties such as tensile strength and elongation at break requires further investigation. Moreover, most of these oils are complex mixtures of different fatty acids, esters, and alcohols, which change with origin. Thus, any formulation derived from them may vary in properties and strength, making unrefined forms difficult and expensive to practically use.

4.6. Coating treatments

Coating treatments play an important role in enhancing aesthetic properties, but also mechanical and functional characteristics of mycelium-based materials. The different types of coatings can help to improve water resistance, durability and flexibility while also influencing the material's visual appearance.

4.6.1. Zein corn

Zein is a class of protein of corn gluten meal and is found in maize. If used as a coating treatment, this protein helps to enhance water resistance, forming a glossy, hydrophobic and yellowish coating. Zein protein is effectively able to form thin films with smooth textures, contributing to improve mechanical integrity of the mycelium material [20]. Additionally, since zein corn is a byproduct of corn production, it serves as a bio-based, non-food competitive component, making it a notable sustainable option for mycelium-based material coatings. Mycoworks, for example, incorporate corn zein in their manufacturing process dissolving the corn zein powder in a solution of warm ethanol and PEG. Once cooled, the solution was applied to the material through dipping, brushing, or spraying. The results suggested that the corn zein coating acts as a moisture barrier, preventing the PEG plasticizer from washing on [22]. Further research is needed to determine the coating's durability through washes, and to optimize the zein corn to plasticizer ratio.

4.6.2. Waxes and Oils

In a recent study by Farrahnoor *et al.* [29], different waxes and oils were investigated for their effectiveness as coating on mycelium mats. The researchers evaluated the impact of waxes, such as beeswax and carnauba wax, and oils, including linseed oil and tung oil, on the performance of mycelium-based materials. It was found that specific combinations of waxes and oils significantly enhanced the hydrophobicity of the materials, making them more suitable for end use. In particular, the combination of beeswax and coconut oil acts to prevent contamination, reduce surface roughness, lower degradation and enhance water repellent properties. Moreover, treatments with oils and waxes were described to improve water resistance, alter texture, and enhance the visual appeal of the mycelium material [25].

4.6.3. Shellac

Shellac is a refined lac resin, a natural biopolymer of animal origin obtained from tiny insects feeding on the sap of specific host trees. Due to its natural, non-toxic, biodegradable, and eco-friendly nature, there is an immense potential to use this material for advanced applications as a replacement for its synthetic counterparts. Shellac, in its basic form, is a polyester macromolecule composed of inter and intra esters of polyhydroxy aliphatic and sesquiterpene acids [30]. In a recent study, Akhter *et al*. [31] reported that applying a surface layer of shellac resin solution enhances the water resistance of mycelium-based vegan leather by reducing water penetration. Additionally, the application of a natural shellac glue solution further minimized the porosity of the material, effectively lowering its water vapor permeability and enhancing the mechanical strength of the mycelium leather.

4.7. Composite Reinforcements

The challenges associated with the tensile strength of mycelium leather-like using various post-treatments can be mitigated using mechanical approaches to textile product development as evidenced by other products on the market. One effective strategy involves incorporating a textile backing to provide additional structural reinforcement, enabling mycelium materials to meet the tensile strength requirements for commercial applications. According to Crawford *et al*. [19], this reinforcement can be integrated during the growth phase, allowing the mycelial hyphae to grow around and fuse with the woven fibers, or after the material has been treated using an adhesive.

4.7.1. Fibrous materials

The term "fibrous material" refers to a material that contains natural fibers, such as hemp, cotton, or linen [27]. For example, Kniep *et al*. [32] in a recent study have integrated rayon fibers to generate materials with higher tensile strength. Rayon is a common regenerated cellulose fiber with good mechanical properties, availability and processability. If fungi can interact with cellulose during the growing process without to metabolize the fibers to prevent to decrease their mechanical strength, then the fibers can be integrated also during the growing mechanism.

4.7.2. Polymers

Some polymers can be used as reinforcement fusion fiber or fibers binder within the fungal mats. Such polymers may be comprised of a synthetic polymer or preferably a natural and/or biodegradable polymer, such as polyhydroxyalkanoates, polyglycolic acid, poly-ε-caprolactone, polycaprolactone, polylactic acid, cellulose acetate, chitin/chitosan, zein corn protein and/or starch [27]. MycoWorks enhances the tensile strength of the mycelium-based materials via lamination of multiple layers and additional surface treatments, including the application of polylactic acid (PLA) coatings [14], [20].

Polylactic acid is one of the most used bio-based and biodegradable thermoplastic polyesters. Its building block monomer, the lactic acid, is produced by the fermentation of carbohydrates such as food wastes, corn stover, and sugar beets. In the study by Chen *et al*. [33], PLA was applied to the surface of mycelium mats using additive manufacturing (3D printing) to create a fungal mycelium-biopolymer composite. By analyzing the mechanical properties after the application of PLA as coating, a significant improvement in ultimate tensile strength was observed, while elasticity was reduced. Specifically, the Young's modulus and ultimate tensile strength increased from 5 ± 2 MPa and 0.6 ± 0.2 MPa (untreated) to 136 ± 58 MPa and 3.1 ± 0.5 MPa (PLA-coated), respectively. The values correspond to an increment factor of ca. 30 for the Young's modulus and of ca. 5 for the ultimate tensile strength, which are a significant rise.

In the study of Madusanka *et al*. [34], polylactic acid was easily applied by first dissolution in water. In this method. The mycelium adsorbs the acid solution, and a PLA film is form on the surface after water evaporation.

Polylactic acid is biosynthesized and can be quickly degraded. Therefore, it is considered a sustainable bioplastic. However, the degradation process is only possible under industrial composting conditions at elevated temperature (higher than 60 °C) because the main depolymerization mechanism is abiotic hydrolysis and not enzymatic degradation [1], [6].

4.7.3. Chitin nanowhiskers

MycoWorks has developed a process to achieve reinforcement by incorporating chitin nanowhiskers into the material [14], [22]. Chitin nanowhiskers are tiny, needle-like structures which can fill the material at microscopic level. The consequent filling reduces the spatial gaps between chitosan fibers of the mycelium mat and reinforces the overall structure. Prior to treatment, the mat undergoes partial deacetylation by immersion in a 40 % w/v sodium hydroxide solution at 80 °C for 1 minute to 10 hours, under precise control of the acetylation degree between 1 % and 50 %. The material is then impregnated with chitin nanowhiskers (~10 nm diameter, 300–500 nm length) via soaking and agitation [3], [14], [17], [22]. The nanowhiskers penetrate the microscopic gaps between hyphae and chitosan chains, forming crosslinking. The result is a nanocomposite mat reinforced through enhanced elastic modulus and tensile strength [10].

4.8. Heat treatment: Hot Pressing

Hot pressing is a key post-processing step used to enhance mechanical, physical, and thermal properties of biomaterials. By carefully controlling the pressing temperature, it is possible to optimize many properties of the biomaterial such as uniformity, homogeneity, isotropy, direction-independent strength, and overall functionality. Table 3 lists the main effects of hot pressing on the mechanical properties of a mycelium-based leather developed with *Formitella Faxinea* mycelium strain. Moreover, hot pressing significantly improves the elastic modulus, making the material stronger and more resilient than when cold pressing is used [2]. Apples *et al*. [24] in their study have noticed an increase in flexural strength when the mat is submitted at hot pressing. In another study, Raman *et al*. [10] have compared some properties of a sample hot pressed and not. The results are summarized in Table 3.

5. CONCLUSIONS

Animal leather is a performant material widely used in clothing, footwear, furniture, and interiors. Despite its potential, the environmental impact of the traditional leather industry has long been a global concern. Therefore, the leather industry is under increasing pressure to reduce its environmental impact. While synthetic leather alternatives derived from plastics typically have a lower carbon footprint than animal hide during production, they rely on fossil resources and are non-biodegradable, raising long-term sustainability concerns. A leading sustainable alternative to both traditional animal leather and synthetic polymer leathers are mycelium-based leather-like materials. To meet consumer expectations for durability, appearance, and functionality, it is essential to focus on post-processing techniques. Chemical, enzymatic, and physical treatments play crucial roles in tailoring the mechanical, thermal, and aesthetic properties of mycelium-based materials. Non-eco-friendly treatments – like chromium and glutaraldehyde– often yield superior mechanical performance, but bio-based alternatives – such as zinc, genipin and tannic acid – offer safer and more sustainable options. The use of biobased plasticizers – like glycerol and sorbitol – can allow flexible materials, without lowering the sustainability of the process. Coating agents – such as corn zein, waxes, oils and shellac – further improve water resistance, and surface finish, broadening the applicability of these materials. Despite their promise, challenges remain in scaling production, reducing costs, and establishing standardized processing methods. Nevertheless, ongoing advances in mycelium cultivation, materials science, and bioengineering are rapidly closing these gaps. With continued innovation, mycelium-based materials have strong potential to become a truly sustainable and functional alternative to conventional leather.

References

[1] Fortune Business Insight (April 2025) [Online]. [Accessed 11.05.2025]. Available: https://www.fortunebusinessinsights.com/leather-goods-market-104405.

[2] Amobonye A., Lalung J., Awasthi M. K., Pillai S. Fungal mycelium as leather alternative: A sustainable biogenic material for the fashion industry. *Sustainable Materials and Technologies* 2023:38:e00724. https://doi.org/10.1016/j.susmat.2023.e00724

[3] Li S., Cao S., Wang X., Zhang Y., Zhang X., Lu W., Zhu D. Investigating the mechanism of Zn cross-linking of chitin in a mycelium-based leather substitute and its performance evaluation. *International Journal of Biological Macromolecules* 2024:276(2):133954. https://doi.org/10.1016/j.ijbiomac.2024.133954

[4] Vandelook S., Elsacker E., Van Wylick A., De Laet L., Peeters E. Current state and future prospects of pure mycelium materials. *Fungal Biology and Biotechnology* 2021:8:20. https://doi.org/10.1186/s40694-021-00128-1

[5] Peeters E., Saluena M., Vandelook S. Growing sustainable materials from filamentous fungi. The *Biochemist* 2023:45(3):8–13. https://doi.org/10.1042/bio_2023_120

[6] D'Errico A., Schröpfer M., Mondschein A., Safeer A. A., Baldus M., Wösten H. A. B. Cross-linking impacts the physical properties of mycelium leather alternatives by targeting hydroxyl groups of polysaccharides and amino groups of proteins. *Heliyon* 2024:10(16):e36263. https://doi.org/10.1016/j.heliyon.2024.e36263

[7] Fuck W. F., Gutterres M., Marcílio N. R., Bordingnon S. The influence of Chromium supplied by tanning and wet finishing processes on the formation of Cr(VI) in leather. *Brazilian Journal of Chemical Eng*ineering 2011:28:221–228. https://doi.org/10.1590/S0104-66322011000200006

[8] Agency for Toxic Substances and Disease Registry (ATSDR) (April 2025) [Online]. [Accessed 11.05.2025]. Available: https://www.atsdr.cdc.gov/

[9] Elsacker E., Vandelook S., Peeters E. Recent technological innovations in mycelium materials as leather substitutes: a patent review. *Frontiers in Bioengineering and Biotechnology* 2023:11:1204861. https://doi.org/10.3389/fbioe.2023.1204861

[10] Raman J., Kim D.-S., Kim H.-S., Oh D.-S., Shin H.-J. Mycofabrication of Mycelium-Based Leather from Brown-Rot Fungi. *Journal of Fungi* 2022:8:317. https://doi.org/10.3390/jof8030317

[11] Shankar M. P., Hamza A., Khalad A., Shanthi G., Kuppireddy S., Kumar D. S. Engineering mushroom mycelium for a greener built environment: Advancements in mycelium-based biocomposites and bioleather. *Food Bioscience* 2024:62:105577. https://doi.org/10.1016/j.fbio.2024.105577

[12] Wiedemann L., Conti F., Sonnleitner M., Saidi A., Goldbrunner M. Investigation and optimization of the mixing in a biogas digester with a laboratory experiment and an artificial model substrate. *EUBCE Proceedings* 2017:889–892. https://doi.org/10.5071/25thEUBCE2017-2CV.4.14

[13] Conti F., Wiedemann L., Sonnleitner M., Goldbrunner M. Thermal behavior of viscosity of aqueous cellulose solutions to emulate biomass in anaerobic digesters. *New Journal of Chemistry* 2018:42(2):1099–1104. https://doi.org/10.1039/C7NJ03199H

[14] Williams E., Cenian K., Golsteijn L., Morris B., Scullin M. L. Life cycle assessment of MycoWorks' Reishi™: the first low-carbon and biodegradable alternative leather. *Environmental Sciences Europe* 2022:34:120. https://doi.org/10.1186/s12302-022-00689-x

[15] Bitting S., Derme T., Lee J., Mele T. V., Dillenburger B., Block P. Challenges and Opportunities in Scaling up Architectural Applications of Mycelium-Based Materials with Digital Fabrication. *Biomimetics* 2022:7(2):44. https://doi.org/10.3390/biomimetics7020044

[16] Huq T., Khan A., Brown D., Dhayagude N., He Z., Ni Y. Sources, production and commercial applications of fungal chitosan: A review. *Journal of Bioresources and Bioproducts* 2022:7(2):85–98. https://doi.org/10.1016/j.jobab.2022.01.002

[17] Kato Y., Kaminaga J., Matsuo R., Isogai A. TEMPO-mediated oxidation of chitin, regenerated chitin and -acetylated chitosan. *Carbohydrate Polymers* 2004:58(4):421–426. https://doi.org/10.1016/j.carbpol.2004.08.011

[18] Olde D. L. H. H., Dijkstra P. J., Van Luyn M. J. A., Van Wachem P. B., Nieuwenhuis P., Feijen J. Glutaraldehyde as a crosslinking agent for collagen-based biomaterials. *Journal of Materials Science: Materials in Medicine* 1995:6:460–472. https://doi.org/10.1007/BF00123371

[19] Crawford A., Ruthanna Miller S., Branco S., Fletcher J., Stefanov D. Growing mycelium leather: a paste substrate approach with post-treatments. *Research Directions: Biotechnology Design* 2024:2:e6. https://doi.org/10.1017/btd.2024.6

[20] Song L., Liu Y., Xiao S., Yuan X., Han X. Revolutionizing Eco-Friendly Leather Production: A Freeze-Thaw and Liquid Fermentation Approach with Fungal Mycelium. *Journal of Fungi* 2025:11:326. https://doi.org/10.3390/jof11040326

[21] Theamdee P., Auasalung T. The Effect of Glycerol Content on Physical and Mechanical Properties of the Biodegradable Film from Sweet Potato Flour for Preserving Namwa Banana. *Life Sciences and Environment Journal* 2019:20:70–80.

[22] Deeg K., Gima Z., Smith A., Stoica O., Tran K. Greener Solutions: Improving performance of mycelium-based leather. *Final Report to MycoWorks* 2017.

[23] Pilz M., Castellan N., Conti F., Qoura F., Brueck T. Sustainable Cultivation of Ascomycete Fungi on Wheat Bran for Hydrolytic Enzyme Production. *Environmental and Climate Technologies* 2024:28(1):510–526. https://doi.org/10.2478/rtuect-2024-0040

[24] Appels F. V. W., Van Den Brandhof J. G., Dijksterhuis J., De Kort G. W., Wösten H. A. B. Fungal mycelium classified in different material families based on glycerol treatment. *Communications Biology* 2020:3:334. https://doi.org/10.1038/s42003-020-1064-4

[25] Wang J., Mutalik R. B., Smith M. J., Subler N. E., McKenzie L., Collins I. S., Flowers K., Addy V., McAusland Bainbridge J., Heinrich M. J. Methods of generating materials with improved properties. U.S. Patent 0007777A1, Jan. 31, 2022.

[26]] Utami Hatmi R., Apriyati E., Cahyaningrum N. Edible coating quality with three types of starch and sorbitol plasticizer. *E3S Web of Conferences* 2020:142:02003. https://doi.org/10.1051/e3sconf/202014202003

[27] Montalti M., Babbini S., Gandia A. Method of producing fungal mats and materials made therefrom. Patent 102018000010869, Jan. 06, 2020.

[28] Naranjo-Briceno L., Fuentes K. M., Escalona G., Rebolledo de Lima H., Figueroa J. M., Zamora P. Nanoemulsion for internal himectation of mycelium-based textiles. Patent WO 040954A1, Feb. 27, 2022.

[29] Farrahnoor A., Sazali N.A.A., Yusoff H., Zhou B. T. Effect of Beeswax and Coconut Oil as Natural Coating Agents on Morphological, Degradation Behaviour, and Water Barrier Properties of Mycelium-Based Composite in Modified Controlled Environment. *Progress in Organic Coatings* 2024:196:108763. https://doi.org/10.1016/j.porgcoat.2024.108763

[30] Thombare N., Kumar S., Kumari U., Sakare P., Yogi R. K., Prasad N., Sharma K. K. Shellac as a multifunctional biopolymer: A review on properties, applications and future potential. *International Journal of Biological Macromolecules* 2022:215:203–223. https://doi.org/10.1016/j.ijbiomac.2022.06.090

[31] Akhter S., Jahan M. S., Rahman M. L., Ruhane T. A., Ahmed M., Khan M. A. Revolutionizing Sustainable Fashion: Jute–Mycelium Vegan Leather Reinforced with Polyhydroxyalkanoate Biopolymer Crosslinking from Novel Bacteria. *Advances in Polymer Technology* 2024:1–10. https://doi.org/10.1155/2024/1304800

[32] Kniep J., Graupner N., Reimer J. J., Müssig J. Mycelium-based biomimetic composite structures as a sustainable leather alternative. *Materials Today Communications* 2024:39:109100. https://doi.org/10.1016/j.mtcomm.2024.109100

[33] Chen H., Klemm S., Dönitz A. G., Ou Y., Schmidt B., Fleck C., Simon U., Völlmecke C. Tailoring the Mechanical Properties of Fungal Mycelium Mats with Material Extrusion Additive Manufacturing of PHBH and PLA Biopolymers. *ACS Omega* 2024:9(50):49609. https://doi.org/10.1021/acsomega.4c07661

[34] Madusanka C., Udayanga D., Nilmini R., Rajapaksha S., Hewawasam C., Manamgoda D., Vasco-Correa J. A review of recent advances in fungal mycelium based composites. *Discover Materials* 2024:4:13. https://doi.org/10.1007/s43939-024-00084-8