Received: 14 October 2024 | Revised: 20 March 2025 | Accepted: 29 April 2025

DOI: 10.1002/wer.70089

RESEARCH ARTICLE

water
ENVIRONMENT RESEARCH

Pure mycelium materials production from agri-processing water: Effects of feedstock composition on material properties for packaging applications

Malvika Sharma[1] | **Livia Fleischmann**[1] | **Maxwell McInnis**[1] | **Arturo Rodriguez-Uribe**[2] | **Manjusri Misra**[1,2] | **Loong-Tak Lim**[3] | **Guneet Kaur**[1]

[1]School of Engineering, University of Guelph, Guelph, Ontario, Canada

[2]Bioproducts Discovery and Development Centre, Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada

[3]Department of Food Science, University of Guelph, Guelph, Ontario, Canada

Correspondence
Guneet Kaur, School of Engineering, University of Guelph, Guelph, Ontario, Canada.
Email: gkaur41@uoguelph.ca

Funding information
Ontario Ministry of Agriculture, Food, and Agribusiness (OMAFA), Grant/Award Number: UGT1-2024-102362

Abstract

In this work, pure mycelium materials (PMMs) were produced by cultivating fungi *Trametes versicolor* and *Irpex lacteus* on lignocellulose-rich agricultural processing water. This water was a side stream from the alkali treatment of purposely grown biomass (miscanthus) for cellulose fiber extraction and contained lignocellulosic residues. Agri-processing water yielded ~75-mg/L PMMs with superior mechano-physical properties than synthetic medium-based ones. These properties were further enhanced by PMM post-processing with glycerol. The thermal stability of PMMs was demonstrated by their higher melting temperature than low density polyethylene (LDPE) while their degradation between 200–380°C, and density of <1.0 g/cm^3, like LDPE. Their mechanical performance was studied on filmlike specimens via dynamic mechanical analyzer. PMMs showed a viscoelastic behavior with a high storage modulus of 34 MPa at 65°C suggesting their suitability for packaging applications. This work provides guidelines on optimizing PMM production using agri-processing water to obtain tunable mechano-physical properties.

Practitioner Points

- Valorization of agri-processing water to produce high-value PMM packaging products.
- No pure or expensive nutrient supplementation was needed for agri-based feedstock.
- Relationships between feedstock composition and PMM properties were established.
- PMMs showed a similar thermal profile and density as typical petro-based packaging materials.
- The addition of glycerol postproduction induced flexibility in PMMs.

KEYWORDS

biomaterials, fungal fermentation, packaging, thermal stability, tunable properties, viscoelastic materials

INTRODUCTION

Petroleum-based polymers play a vital role in the packaging industry where these are used in rigid containers, films, foamed containers, and pouches, to contain, protect, and transport products (Stark & Matuana, 2021). However, their fossil fuel origin, manufacturing methods, and lack of sustainable end-of-life options lead to significant environmental impacts, including single-use plastics (SUPs) pollution and greenhouse gases emissions, thereby raising concerns on their packaging applications (Haneef et al., 2017). These concerns have led several countries to prohibit the use of petrochemically derived SUP-based packaging materials for food-based applications, which is one of the major users of SUP packaging (Cruz et al., 2022; Environment and Climate Change Canada, 2022). These challenges have driven research towards the production of sustainable alternatives such as biopolymers (or biomaterials) and their applications in food and other packaging industries.

Despite significant advancements in sustainable material development, current alternatives to packaging materials often fall short in terms of ease of manufacturing and environmental impact. Bio-based materials like polymers from natural sources such as cellulose (Sharma et al., 2022), pectin (Guo et al., 2021), and microbial sourced polymers like bacterial cellulose (Wang et al., 2019) require complicated and time-intensive methods of processing, extraction, washing with hot solutions of harsh chemicals like sodium hydroxide (NaOH). Microbially derived polyhydroxyalkanoates, being intracellular products, pose difficulties in downstream processing, making their production time-consuming and challenging (Joyline & Aruna, 2019). Additionally, some bioplastics like poly(lactic acid) (PLA) only degrade under industrial composting conditions with elevated temperatures (>60°C), which begs the question on biodegradability (Husárová et al., 2014). As a viable solution to these limitations, developing mycelium-based biomaterials presents a promising approach. These biomaterials can be produced quickly within 2–3 weeks, depending on the fungal species and feedstock used, and without costly and complex processing methods, thus providing a more sustainable and practical alternative. Fungal hyphae form a macroscopic network known as mycelium, which has garnered significant interest from researchers and industry as an emerging sustainable packaging biomaterial. Composite mycelium materials (CMM) are one such renewable biomaterial substitutes produced by filling the empty spaces of lignocellulosic waste feedstock with mycelium (Peeters et al., 2023; Rafiee et al., 2021). Mycelium acts as a biological glue, which binds the loose particles in the feedstock, forming a composite material shaped by the mold. These materials exhibit foamlike properties, similar to expanded polystyrene, making them suitable for various applications like packaging and insulation (Saini et al., 2024.; Sydor et al., 2021). However, the properties of these materials are restricted by the mold shape and the lignocellulosic waste substrates used. In contrast, pure mycelium materials (PMMs) are devoid of residual feedstock particles, and their properties are determined by the fungal organism. Furthermore, the final material exhibits characteristics that are influenced by the fungal growth conditions. Fungi are highly responsive to these conditions which shape their dynamic cell wall structure, and the latter can be exploited to endow PMMs with foam, leather, paper to polymerlike characteristics (Elsacker et al., 2023; Peeters et al., 2023; Vandelook et al., 2021). This design flexibility offers diverse industrial applications of PMMs. For example, within the food and beverage sector, PMMs can be used to make different types of packaging products such as food containers, lids, film wraps, and carry bags, which have varying degrees of elasticity and rigidity, and potentially provide a suitable alternative to SUP packaging materials, such as poly(ethylene terephthalate) (e.g., food containers and bottles) and low density polyethylene (LDPE) (e.g., wraps) (Shin et al., 2016; Welle, 2011). Moreover, PMMs can be functionalized by appropriate growth and/or post-processing methods, which could further enhance their natural hydrophobic and antimicrobial properties and provide food safety (Nasir & Othman, 2020).

Identification of optimum growth conditions producing target PMM properties can be achieved by understanding the interrelationships among environmental conditions and the properties of the resulting biomaterial by variation in hyphal structure and their orientation, and cell wall composition. Currently, there is limited information and a lack of understanding on how changes in these conditions impact the properties of the final biomaterial (Peeters et al., 2023). Another major challenge lies in developing cost-effective and high-yielding processes, which can be addressed through

strain selection and optimization based on the composition and availability of feedstocks. The present study investigated how different feedstocks, including synthetic media and lignocellulosic-rich agri-processing water, influenced the properties of PMMs produced by different fungi. The agri-processing water is defined as effluents or wastewater generated as side streams (byproducts) during the processing of agricultural products such as miscanthus, olive oil, palm oil, and whole rice. Being rich in nutrients, these could make good feedstocks for bioprocesses. This research used the wastewater arising from the alkali treatment processing of purposely grown biomass (miscanthus) for cellulose fiber extraction. The resulting nutrient-rich water containing lignin, remaining cellulose, and hemicellulose was used in the present study for PMM production. To the best of authors' knowledge, this is the first report on the valorization of such lignocellulosic-rich water streams, wherein these are used as sole feedstock to produce PMM and characterization of their properties for packaging applications is performed. By systematically comparing various substrates, we aim to identify the specific combinations of nutrients and fungal strains to produce PMM with different production yields and properties. This understanding would enable the selection of the most suitable feedstock for targeted applications and contribute to the development of a more sustainable and economically viable production process for PMM. Furthermore, the effect of plasticizers on the material properties of agri-processing water-derived PMMs is investigated in the present work. Results from this study would facilitate the design of process engineering strategies for obtaining high yields of PMM with desirable properties using low-value feedstock, which currently limits their market applications.

MATERIALS AND METHODS

Strain and preparation of feedstock

Trametes versicolor and *Irpex lacteus* mushrooms were sourced from Happy Caps Mushroom Farm, Nova Scotia, Canada. The mycelia were maintained in 10 × 15-mm Petri dishes with malt extract agar (MEA, Oxoid Ltd., UK) containing 30-g/L malt extract, 5-g/L mycological peptone, and 15-g/L agar as the growth medium. Strain identification was performed using 16S/18S rRNA sequencing of the isolated and agar-maintained mycelia by Agriculture and Food Laboratory, University of Guelph. The obtained 16S and 18S rRNA genes were amplified using specific primers in a polymerase chain reaction (PCR). The amplifications from PCR were loaded on a gel for gel electrophoresis to achieve the bands for strain identification. Only sequences with a similarity percentage of 99% or above were considered. The identified strains, found to be *Trametes versicolor* and *Irpex lacteus*, were further cultured in potato dextrose broth (PDB, Becton, Dickinson and Company, USA), malt extract broth (MEB, Becton, Dickinson and Company, USA), and lignocellulosic-rich agricultural water. The agri-processing water used in this study consisted of side stream arising from alkali treatment processing of purposely cultivated miscanthus biomass by DMT Bioproducts, Aylmer, Canada. It contained lignin, remaining cellulose, and hemicellulose and had the characteristics of total organic carbon 13.25%, total nitrogen 0.162-g/L, and pH 7.1. This processing water was used as a sole feedstock for PMM production and without any pure and expensive nutrient supplementation. The feedstock was used as it is or diluted with water based on mycelial growth observed under various dilutions. Both the synthetic media and agri-processing water were autoclaved before use. Furthermore, with an aim to develop a cost-effective process, experiments were also performed with a non-autoclaved agri-processing water-based feedstock to produce PMMs without sterilization. In all cases, the pH of agri-processing water was adjusted to pH 4.8 using 2-M HCl from an initial pH of 7.1 to provide optimal pH conditions for mycelium growth and PMM production for which the ideal range is between 4.5 and 5 (Veena & Pandey, 2012).

Production conditions of PMMs on various feedstocks

A 150-mL volume of various feedstocks was inoculated with a 1 × 1-cm mycelium disc obtained on malt extract agar (either *T. versicolor* or *I. lacteus*) in a 500-mL conical flask, which was covered with aluminum foil. The inoculated media were then incubated (New Brunswick, USA) at 25°C under static conditions and mycelium grown under closed flask conditions. The fermentation was conducted as a batch, and no additional medium was added to the flasks during cultivation. The cultivation period varied depending on the substrate: 10 days for *T. versicolor* and 14 days for *I. lacteus* in synthetic media, and 28 days for agri-processing water feedstock. This variation was due to the differences in feedstock composition, with the agri-processing water requiring more time for the cultures to acclimatize to complex organic compounds in them, as observed in our preliminary experiments (data not shown). After PMM growth was obtained, the mycelium was harvested and washed with deionized water. The fungal biomass was tapped dried on

a Whatman filter paper (150-mm diameter, Fisher Scientific, Canada) to remove excess medium, placed between two sheets of parchment papers, and then sandwiched between two stainless steel meshes covered with aluminum foil. The sandwiched samples were compressed under a 2-kg weight (to avoid shrinkage) at room temperature ($22 \pm 2°C$) to form dried mycelium sheeting (Wijayarathna et al., 2022). This was done until a constant weight was achieved.

The wet weight of PMM was recorded using an analytical balance immediately after harvesting the samples from various feedstocks. The dry weight was measured after drying the samples at room temperature.

Post-processing treatment of PMMs

Upon drying, PMMs exhibited brittle characteristics, therefore with an aim to enhance their flexibility; the synthesized PMMs were treated with 10% and 30% v/v glycerol concentrations. For this, dried PMMs were submerged in glycerol for 24 h at room temperature ($22 \pm 2°C$). Following this, dried mycelium sheeting was obtained again in the same manner as described above. Since *I. lacteus* produced a higher dry weight than *T. versicolor*, only the *I. lacteus*-derived agri-processing water-derived PMMs were subjected to glycerol treatment and further characterized.

Characterization of PMMs

Chemical characterization of PMMs

Fourier transform infrared (FTIR) spectroscopy was employed to examine how the variations in the feedstock composition affected the components of synthesized PMM. The analysis was conducted in an attenuated total reflectance (ATR) mode using an FTIR spectrophotometer (PerkinElmer, Spectrum 4000 series, USA). Infrared spectra were collected within the range of 4000 to 800 cm^{-1}, with a resolution of 4 cm^{-1}. An average of 100 scans was performed for each determination (Bustillos et al., 2020; Haneef et al., 2017). The spectra obtained were then analyzed using Origin Pro software (Version 9, Origin Lab Corporation, MA, USA).

Thermal profile analysis of PMMs

Thermogravimetric analysis (TGA) was used to investigate the thermal stability of PMMs by analyzing the change in mass with temperature. Studies were performed using TA instrument (SDT-Q600, TA Instruments-Waters LLC, New Castle, USA) with a temperature range of 25 to 600°C and a heat flow rate of 10°C/min under nitrogen atmosphere. The thermal profile data were then analyzed using Origin Pro software (Version 9, Origin Lab Corporation, MA, USA).

The thermal behavior of PMMs during heating was analyzed using differential scanning calorimetry (DSC) (model Q2000, TA instruments, New Castle, USA). Samples weighing 5–10 mg were placed in hermetically sealed aluminum DSC pan and heated from 22 to 300°C at a rate of 5°C/min under nitrogen atmosphere. The glass transition temperature (T_g) and melting temperature (T_m) were determined at the midpoint of the observed endothermic peaks. DSC thermograms were analyzed using TA Universal Analysis Software (TA Instruments, New Castle, USA).

Morphological characterization of PMMs

Scanning electron microscopy (SEM) (Inspect S50, FEI Corp., Hillsboro, Oregon, USA) was employed to determine the morphology of the synthesized PMM with 20-kV acceleration voltage. Samples were mounted on aluminum stubs using two-sided carbon tape followed by sputter-coating with gold, to prevent charging, before the analysis.

Density measurement of PMMs

The density of the synthesized PMM was determined using an electronic densimeter (MD-300S, Alfa Mirage Co. Ltd., Osaka, Japan). At least three replicates were used for measurements. Average values and the corresponding standard deviations were calculated and reported.

Mechanical properties analysis of PMMs

Dynamic mechanical analysis (DMA) was used to determine the storage modulus (E′), loss modulus (E″) and loss tangent (tan δ) of PMM samples (model Q800 DMA, TA instruments, USA). Samples were cut into 3 × 1-cm rectangle and were tested from room temperature (25°C) to 80°C in a uniaxial tensile mode with a heating ramp of 2°C/min. Sinusoidal deformations were applied with an amplitude of 20-μm and 1-Hz frequency. The untreated PMMs were not analyzed due to their brittleness. Therefore, the results for only the posttreated PMMs with glycerol are shown.

Statistical analysis

Two-way Analysis of Variance (ANOVA) was performed to statistically analyze the data. The analysis was performed using Minitab Statistical Software, version 22 (Minitab, LLC, Pennsylvania, USA). For mycelium growth analysis, the processing conditions (autoclaved or non-autoclaved) and fungal type (*T. versicolor* and *I. lacteus*) were considered as independent variables and growth parameters (wet weight and dry weight) as dependent variables.

RESULTS AND DISCUSSION

Growth of PMMs

Preliminary experiments using agri-processing water as feedstock demonstrated that it was difficult for the fungi used in this study to grow and produce PMM on the agri-processing water feedstock at 50% and 100% v/v concentration levels. However, reducing the concentration to 10% v/v supported mycelium growth. The key factor influencing fungal growth appeared to be the presence of inhibitory compounds in the agri-processing water which we had used in this study. It contained some inhibitory compounds, such as phenols and fluoride. These compounds have been shown to significantly impact fungal growth and metabolism, when present even at low concentrations of mg/L level, especially the fluoride salts (Agrawal et al., 1983; El-Zaher et al., 2011; Türkkan & Erper, 2014). In the present study, when lower feedstock concentrations (10% v/v) were used, the dilution of these inhibitory substances likely reduced their inhibitory effects, allowing the fungi to grow and produce PMMs. However, at higher concentrations (50%–100% feedstock), these compounds could reach the growth inhibitory levels.

Figure 1a illustrates the wet weight of PMM produced by *T. versicolor* and *I. lacteus* on 10% agri-processing water feedstock. *T. versicolor* outperformed *I. lacteus*, producing approximately 2.6-fold more PMM on non-autoclaved feedstock and 2.4-fold more on autoclaved feedstock. These results highlighted the superior efficiency of *T. versicolor* in producing PMM. However, statistical analysis indicated that the autoclave condition (autoclaved or non-autoclaved) had no significant effect on mycelium growth ($p > 0.05$), while the fungal type, *T. versicolor* versus *I. lacteus* significantly affected the growth (wet weight) ($p < 0.05$). Additionally, the type of fungi also significantly influenced dry weight ($p < 0.05$), whereas the processing condition showed no significant effect ($p > 0.05$) (Figure 1b). Although *I. lacteus* demonstrated higher dry weight production on the autoclaved feedstock, the difference was statistically insignificant ($p > 0.05$), suggesting that this observed variation in growth may not be directly attributable to the processing condition. On the non-autoclaved feedstock, the difference in dry weight production between the two fungal types decreased, with *I. lacteus* producing only 1.2 times more PMM than *T. versicolor*. However, this difference was statistically insignificant ($p > 0.05$). These findings suggest that agri-processing water can support the growth of both fungi, *T. versicolor* and *I. lacteus* as a sole nutrient

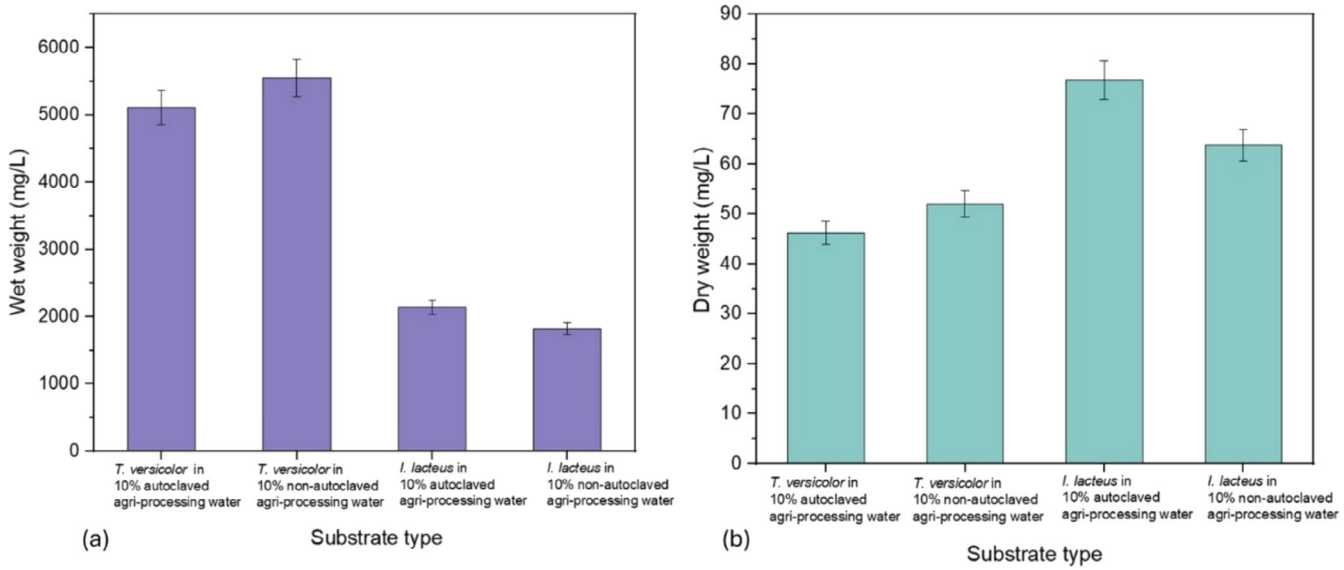

FIGURE 1 **Production of pure mycelium materials (PMM) by *T. versicolor* and *I. lacteus* in 10% agri-processing water.** Analysis of (a) wet weight and (b) dry weight of materials is shown.

source without the requirement of any nutrient supplementation.

Lignin content may play a role in fungal growth while other factors such as type of fungi also critically influence PMM yield. The growth of PMM began with a 1 × 1-cm mycelial disc as the inoculum. In synthetic media, both fungi exhibited rapid growth with notable differences in their growth patterns. *T. versicolor* initially expanded from the inoculum, progressively forming a larger spot that developed into a sheet covering the entire surface of the medium (at air-liquid interface) within 10 days. In contrast, *I. lacteus* grew as a single expanding spot, spreading the mycelium throughout the substrate and forming a sheet at the surface over the 10-day period. The mycelium growth on different feedstocks is illustrated in Figure 2. This accelerated growth of *T. versicolor* compared to *I. lacteus* might be due to the efficient incorporation of these simple nutrient components into the respiratory pathway. As similar growth proliferation was also reported for *Pleurotus pulmonarius* mycelial biomass grown on various carbon sources such as glucose, xylose, galactose, and arabinose (Smiderle et al., 2012). A slow growth pattern was observed for both fungi in agri-processing water feedstock, which can be attributed to the complexity of the feedstock composition. The mycelium of both fungal types grew on the feedstock over a 28-day period without the formation of a sheet on the surface (air–liquid interface) and was subsequently harvested. This harvested fungal biomass was formed into a thin sheet and the synthesized PMMs post-drying are shown in Figure 3. Most studies have focused on producing PMMs using synthetic growth media. The production of these media is energy- and resource-intensive, and a time-consuming process requiring the growth of sources like potato and barley, followed by processing conditions such as extraction and drying to produce powdered media. While the utilization of such expensive feedstock for PMM production has been largely reported, it does not align with the principles of circular economy and undermines the development of sustainable bio-based products (Antinori et al., 2020; Haneef et al., 2017; Rathinamoorthy et al., 2023). On the contrary, the present work is the first report to demonstrate the production of PMM solely on nutrient-rich agri-processing water-based feedstock, which is obtained as a waste stream from biomass processing. This is a significant result since it opens avenues for bioprocess optimization and large-scale process development to realize the potential applications of PMM.

The growth analysis showed that wet and dry mycelial weights were significantly higher in synthetic media (PDB and MEB) due to the simpler nutrient compounds that support energy production and growth, compared to agri-processing water feedstock. Lignin binds to hemicellulose and cellulose, which are the primary carbon and energy sources for these fungi (Xiao et al., 2017). The degradation of lignin by oxidative and hydrolytic enzymes is necessary to access these energy sources (Fernández-Fueyo et al., 2016; Wan & Li, 2012). Fungi produce several enzymes, such as laccase, manganese peroxidase, lignin peroxidase, and versatile peroxidase, to break down lignin and access the nutrients in lignocellulosic biomasses (Kumar & Chandra, 2020; Qin et al., 2018). In environments rich in lignin, fungal growth is limited due to the reduced availability of carbon and nitrogen sources. This explains the rapid mycelial growth within

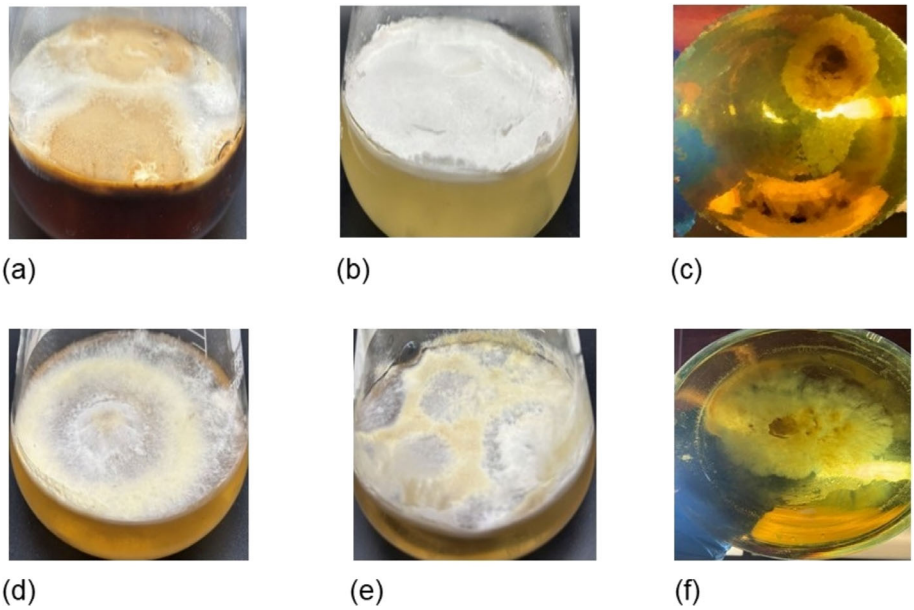

FIGURE 2 Growth of various fungi on different feedstocks to produce pure mycelium materials (PMM). (a) *T. versicolor* cultivated in PDB; (b) *T. versicolor* cultivated in MEB; (c) *T. versicolor* cultivated in 10% agri-processing water-based feedstock; (d) *I. lacteus* cultivated in PDB; (e) *I. lacteus* cultivated in MEB; (f) *I. lacteus* cultivated in 10% agri-processing water-based feedstock. Note the surface (air–water interface) growth of both fungi on synthetic media (a,b,d, e) and growth at the bottom of the flask on agri-processing water-based feedstock (c,f).

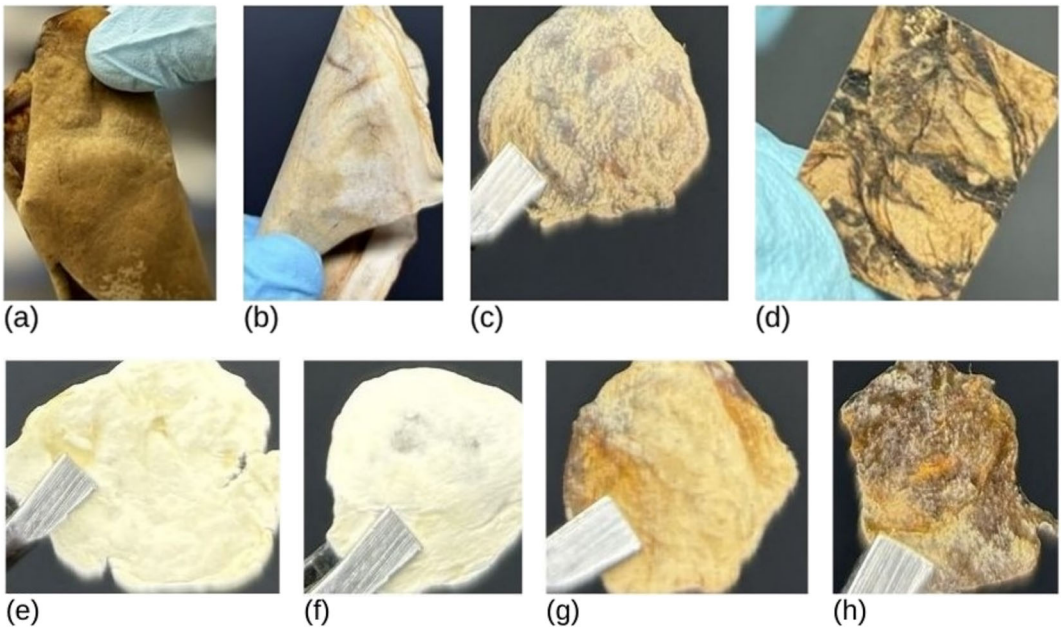

FIGURE 3 **Bio-fabricated pure mycelium material (PMM) sheets obtained from:** (a) *T. versicolor* cultivated in PDB; (b) *T. versicolor* cultivated in MEB; (c) *T. versicolor* cultivated in 10% autoclaved agri-processing water-based feedstock; (d) *T. versicolor* cultivated in 10% non-autoclaved agri-processing water-based feedstock; (e) *I. lacteus* cultivated in PDB; (f) *I. lacteus* cultivated in MEB; (g) *I. lacteus* cultivated in 10% autoclaved agri-processing water-based feedstock; and (h) *I. lacteus* cultivated in 10% non-autoclaved agri-processing water-based feedstock.

10 or 14 days in the synthetic media, where nutrients are more readily available than in the agri-processing water. On the latter, visible threadlike structures appeared on the mycelium inoculum disc from Day 3 of inoculation. After this stage, rapid mycelium growth was observed at the bottom of the flask, which continued until 28 days. This fast growth could be attributed to the release of fungal lignocellulolytic enzymes required to assimilate nutrients. The fungi (*T. versicolor* and *I. lacteus*) used in this study secrete a variety of lignocellulolytic enzymes, including manganese peroxidase (Duan et al., 2018), lignin peroxidase (Chen et al., 2022), and laccase (Iqbal et al., 2011), which facilitate the degradation of lignin, cellulose, and hemicellulose. This unique characteristic makes these white rot fungi a crucial component in degrading lignocellulosic-rich biomass. This capability is not as prevalent in other fungi, such as the brown rot fungi (Duan et al., 2018) and may explain why *I. lacteus* was able to grow on the agri-processing water used in the present study.

Chemical characterization of PMMs

The ATR-FTIR analysis of PMMs derived from different feedstocks and fungi used in this study revealed the presence of similar chemical components, although with variations in their respective absorption intensities. As shown in Figure 4, the spectra indicated consistent functional groups across all samples, suggesting a shared biochemical composition influenced by the feedstock type. The absorption bands in the region 3600–3000 cm^{-1} corresponded to OH stretching, while those in the 3000–2900-cm^{-1} region were correlated to CH stretching, indicative of several components such as polysaccharides, chitin, and proteins, which are naturally present in the fungal cell wall (Bustillos et al., 2020; Haneef et al., 2017). Additionally, absorption peaks between 1700 and 1450 cm^{-1} were associated with the presence of C=O stretching (amide I) and N-H deformation (amide II) (Antinori et al., 2021; Bustillos et al., 2020). It was observed that both the strains showed the presence of C=O stretching and N-H deformation corresponding to amide I and amide II bonds, respectively, across all the feedstocks but with variable absorption intensities. For example, in PMM derived from *T. versicolor* grown in synthetic media, medium-intensity amide I peaks were observed at 1650 cm^{-1} for PDB and 1643 cm^{-1} for MEB. However, when grown on agri-processing water, the intensity of these peaks weakened. A similar trend was observed for amide II-related bands with absorption bands observed at 1542 cm^{-1} (PDB), 1543 cm^{-1} (MEB), 1548 cm^{-1} (10% autoclaved agri-processing water), and at 1542 cm^{-1} (10% non-autoclaved agri-processing water).

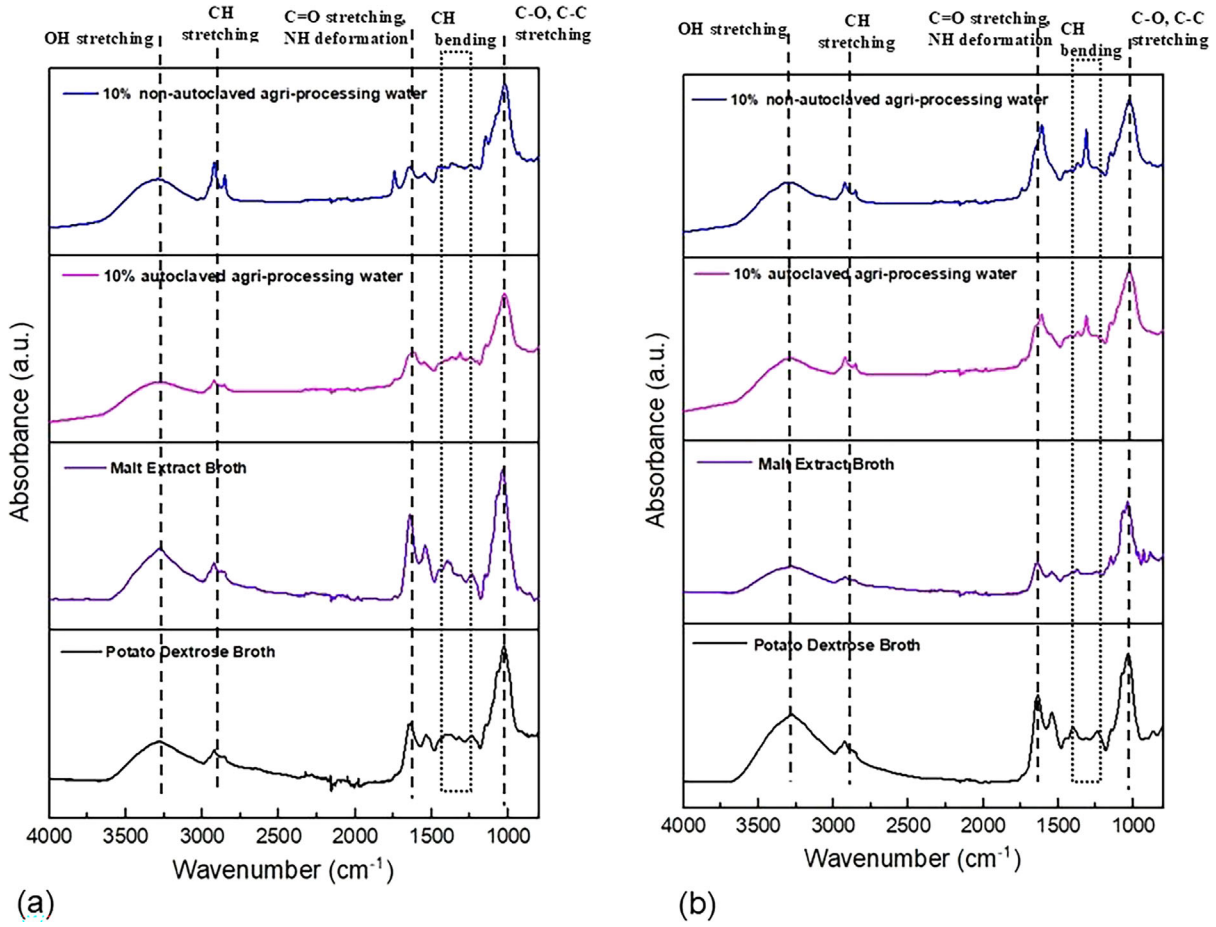

FIGURE 4 Attenuated total reflectance (ATR)-Fourier transform infrared (FTIR) spectra of pure mycelium materials (PMM) grown on various feedstocks. (a) Infrared spectra of *T. versicolor* PMM on synthetic medium and 10% agri-processing water-based feedstock; and (b) infrared spectra of *I. lacteus* PMM on synthetic medium and 10% agri-processing water-based feedstock.

The presence of these bands could be related to the presence of proteins in the fungal cell wall. The absorption bands in the region 1200–750 cm^{-1} corresponded to C–C stretching and C–O stretching absorption bands of polysaccharides present in the cell wall (Peltzer et al., 2018; Pilafidis et al., 2024). Strong intensity absorption bands at 1028 and 1038 cm^{-1} were correlated to PMM derived from *T. versicolor* grown on PDB and MEB, respectively. In contrast, medium-intensity absorption bands appeared at 1024 and 1021 cm^{-1} were observed for 10% autoclaved agri-processing water and 10% non-autoclaved agri-processing water, respectively. Additionally, weak intensity absorption bands at 1370 cm^{-1} were also observed corresponding to CH bending, which may be associated with chitin (Khamrai et al., 2018). The presence of medium-intensity bands in region 1700 to 1450 cm^{-1} and strong intensity bands in region 1200–750 cm^{-1} for PMM derived from synthetic media, compared to agri-processing water, suggested variations in biochemical composition, likely influenced by differences in nutrient availability. The simpler and defined composition of synthetic media may facilitate a more consistent metabolic response in *T. versicolor*, potentially leading to increased production of certain macromolecules such as proteins and polysaccharides, as reflected in the observed FTIR spectra.

The ATR-FTIR spectra analysis of *I. lactues*-based PMM revealed the presence of absorption bands around 3600–3000 cm^{-1}, which corresponded to OH stretching, while the bands in the 3000–2900 cm^{-1} region were attributed to CH stretching, both of which are associated with the presence of polysaccharides, chitin, and proteins, as discussed previously. The amide I (C=O stretching) and amide II (N-H deformation) bands were observed in the 1700–1450-cm^{-1} region, with specific peaks recorded at 1635 and 1546 cm^{-1} for PMM derived from PDB, 1635 and 1543 cm^{-1} for MEB, 1616 cm^{-1} for 10% autoclaved agri-processing water, and 1613 cm^{-1} for 10% non-autoclaved agri-processing water. It was observed that PMM grown in synthetic media exhibited medium-intensity absorption bands for both amide I and amide II regions, whereas for PMM grown in

agri-processing water, amide I bands remained at medium intensity, while amide II bands weakened. In the 1200–750-cm^{-1} region, medium-intensity absorption bands at 1033, 1043, and 1028 cm^{-1} were correlated with C–C and C–O stretching of polysaccharides. Additionally, weak absorption bands at 1370 cm^{-1} were present in synthetic media-derived PMM, whereas medium-intensity bands were observed in agri-processing water-derived PMM, possible attributed to the presence of chitin.

These results suggested that while *I. lacteus* exhibited comparable cell wall composition across different feedstocks, the biochemical profile varied in response to nutrient availability. The weaker intensity of amide II bands in agri-processing water-based PMM indicated a possible reduction in protein content or differences in fungal metabolism under these conditions. Similarly, the stronger absorption at 1370 cm^{-1} in agri-processing water-derived PMM may suggest variations in chitin content, potentially influenced by the complexity of this feedstock.

The PMM samples were posttreated by submersion in glycerol to enhance the physico-mechanical properties. Glycerol is a by-product of biodiesel industry and is Generally Recognized as Safe (GRAS) for use in the food packaging industry (Ben et al., 2022). Posttreatment of *I. lacteus*-based PMMs grown on different feedstocks resulted in significant changes in the ATR-FTIR spectra as shown in Figure 5, indicating interactions between the fungal cell wall components and glycerol. For example, a shift in wavenumber from 3276 cm^{-1} in PDB-based

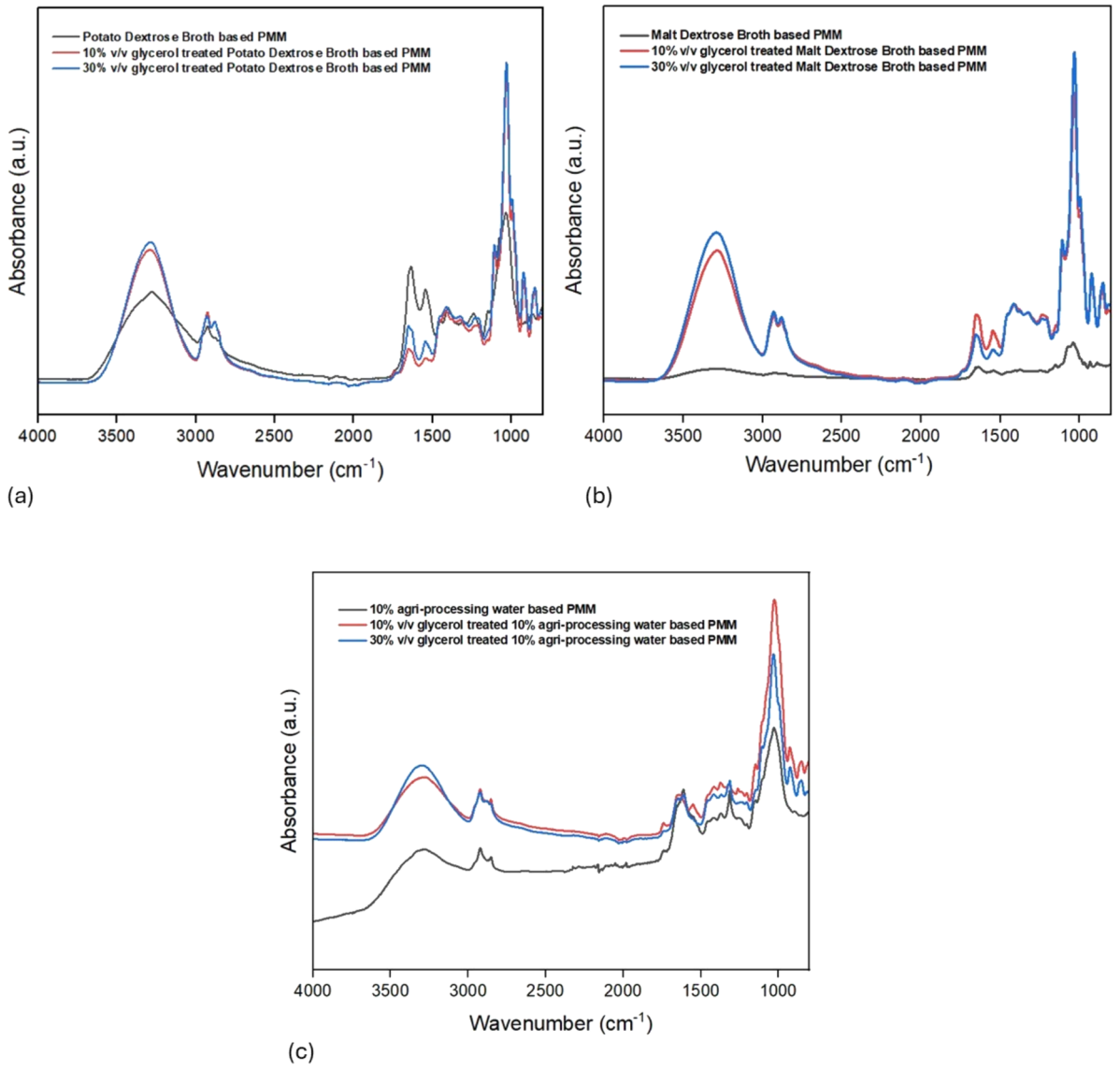

FIGURE 5 Attenuated total reflectance (ATR)-Fourier transform infrared (FTIR) spectra of glycerol-treated pure mycelium materials (PMM) grown on various feedstocks. (a) Infrared spectra of *I. lacteus* PMM on PDB; (b) infrared spectra of *I. lacteus* PMM on MEB; and (c) infrared spectra of *I. lacteus* PMM on 10% agri-processing water-based feedstock.

PMM to 3289 and 3281 cm^{-1} was observed upon treatment with 10% v/v and 30% v/v glycerol, respectively. A similar shift to higher wavenumbers was observed in PMMs developed from MEB and 10% agri-processing water, further suggesting the interaction. A corresponding trend was noted for the absorption bands in 1200–750-cm^{-1} region, which may be attributed to C–OH stretching due to the presence of glycerol in plasticized PMMs, while in non-plasticized PMMs, it could be associated with C–C and C–O stretching of polysaccharides, as previously discussed (Peltzer et al., 2018). It was also observed that the absorption bands of amide I (C=O stretching) and amide II (N-H deformation) shifted to a higher wavenumber for PMM grown on MEB and 10% agri-processing water. This shift may be attributed to the interactions between the hydroxyl group of glycerol (acting as a hydrogen donor) and the oxygen atoms in the hydroxyl group of chitin and amide group (both serving as hydrogen bond acceptors) (Guerrero et al., 2023). Conversely, a shift to lower wavenumber with lower absorption intensity was observed in plasticized PDB-based PMM. This may result from the lower protein content in plasticized samples, as well as the interaction of the N-H group with glycerol, given its high sensitivity to hydrogen bonding, which can alter its conformation (Gao et al., 2006). A comparable shift to lower wavenumbers was also reported by Peltzer et al. (2018) during the analysis of yeast-based biomaterials. The observed differences in chemical nature of PMMs upon plasticizer treatment provide a means to altering their physico-mechanical properties, as further discussed below.

Thermal profile analysis of PMMs

The thermal degradation behavior of PMM produced by *T. versicolor* and *I. lacteus* on agri-processing water feedstock was analyzed using TGA, as shown in Figure 6a,b, respectively. Table 1 presents the mass loss data from the TGA analysis, indicating that all the produced materials exhibited comparable mass loss percentages. These analyses offer insights into the melting and degradation temperatures of materials, aiding in the selection of the optimal processing temperatures for manufacturing techniques like injection molding or extrusion, which are used for the production of different types of packaging products, for example, wraps and food containers (Basil et al., 2023).

In the present study, TGA and DTG plots revealed three distinct stages of mass loss. The first stage, occurring

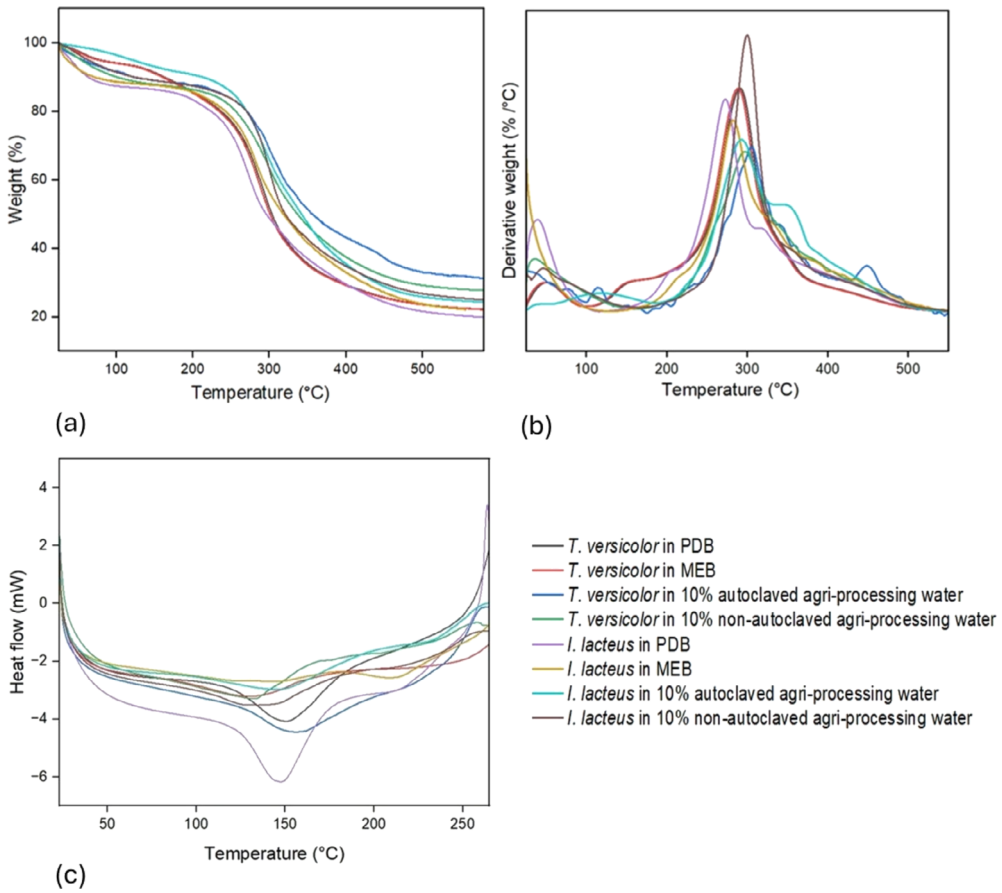

FIGURE 6 Thermal characterization of pure mycelium materials (PMM). (a) Thermogravimetric analysis (TGA); (b) derivative thermogravimetric (DTG); and (c) differential scanning calorimetry (DSC) curves of PMM synthesized by *T. versicolor* and *I. Lacteus* on various feedstocks.

TABLE 1 Mass loss (%) of pure mycelium materials (PMM) as determined from thermogravimetric analysis (TGA).

PMM type	Mass loss (%)
T. versicolor in PDB	77.61
T. versicolor in MEB	77.54
T. versicolor in 10% autoclaved agri-processing water	68.25
T. versicolor in 10% non-autoclaved agri-processing water	72.17
I. lacteus in PDB	79.16
I. lacteus in MEB	76.51
I. lacteus in 10% autoclaved agri-processing water	75.37
I. lacteus in 10% non-autoclaved agri-processing water	74.92

between 25 and 200°C, was associated with water evaporation (Wijayarathna et al., 2022). The second stage, from 200 to 380°C, involved the degradation of organic compounds such as chitin, proteins, and polysaccharides, which formed the structural components of the mycelium. This stage also included the dehydration of saccharide rings and the breakdown of acetylated and deacetylated chitin units (Rathinamoorthy et al., 2023). The final stage of degradation occurred beyond 380°C and was linked to the decomposition of residual char from the combustion of mycelium. Similar results were observed for PMM produced on synthetic media. The thermal profiles were nearly identical to those of petroleum-based plastics and bio-based packaging films. According to Sorolla-Rosario et al. (2022), LDPE exhibited a complete degradation beyond 400°C. Similarly, Sharma et al. (2022) reported the thermal degradation ranges for bio-based films made from starch and microfibrillated cellulose fibers, spanning from 200 to 350 and 380–450°C. PMMs produced in this study demonstrated a comparable thermal stability to currently existing packaging materials such as LDPE and other bio-based alternatives.

DSC analysis was conducted on PMMs to assess key thermal properties, particularly melting temperatures, which are critical for packaging applications. The results for PMMs derived from *T. versicolor* and *I. lacteus* cultivated on various feedstocks are presented in Figure 6c. Based on the DSC curve, the first melting temperature (T_m1) ranged from 134 to 157°C, corresponding to the denaturation of proteins within the PMM (Xia et al., 2023). A subsequent endothermic peak (T_m2) was observed between 212 and 252°C, indicating the denaturation of the chitin-glucan complex and β-chitin, as also reported by Jang et al. (2004) and Farinha et al. (2015). The higher thermal stability of chitin and glucan, compared to proteins, suggests their significant contribution to the material's overall thermal resistance. The ATR-FTIR results (Section 3.2) confirmed the presence of these components in the PMM. As observed, the differences in melting temperatures are minimal, indicating that the different feedstocks used for PMM production had minimal impact on the thermal stability of the materials. In comparison, bioplastic PLA exhibited two melting temperatures at 124 and 161.1°C, as reported by Yu et al. (2020) while LDPE was reported to have a T_m of 109°C (Sorolla-Rosario et al., 2022). The higher melting temperatures and denaturation points observed for the PMM in this study indicated their enhanced thermal stability compared to conventional petroleum-based polymers as well as bio-based polymers like PLA, making PMM a promising material for packaging applications requiring higher thermal resistance.

Morphological characteristics of PMMs

The surface morphology of the synthesized PMM was examined using SEM and is shown in Figure 7. The analysis revealed that all PMMs featured a network of randomly oriented, entangled, unbranched, and either flat or tubelike filamentous structures. They lacked septa within the hyphal structure, which can contribute to their stability under mechanical stress (Bustillos et al., 2020; Rathinamoorthy et al., 2023). PMM produced by *T. versicolor* in PDB and MEB displayed short to long, intertwined, and threadlike structures, with hyphal diameters of 2.32 ± 0.20 μm in PDB and 2.33 ± 0.06 μm in MEB. In contrast, *I. lacteus* produced highly tangled, short, and tubelike structures, with hyphal diameters of 2.62 ± 0.4 μm in PDB and 2.2 ± 0.2 μm in MEB. The shorter hyphal configurations in *I. lacteus* may be due to its slower growth in synthetic media, resulting from moderate nutrient metabolism over 14 days. Synthetic media did not significantly affect the hyphal diameter for either strain. PMM grown on lignin substrates exhibited closely stacked, less porous, and flatter surface morphologies, with smoother and less tangled hyphal networks compared to those grown in synthetic media. The average hyphal diameter for PMM grown on agri-processing water feedstock was 2.52 ± 0.45 μm for *T. versicolor* and 2.7 ± 0.2 μm for *I. lacteus*.

A modification in surface morphology was observed in PMMs upon glycerol treatment. The treated PMMs were smoother and less porous than the untreated ones, likely due to the penetration of glycerol into the voids of the treated PMMs. This infiltration lowered the surface roughness and promoted the formation of intermolecular

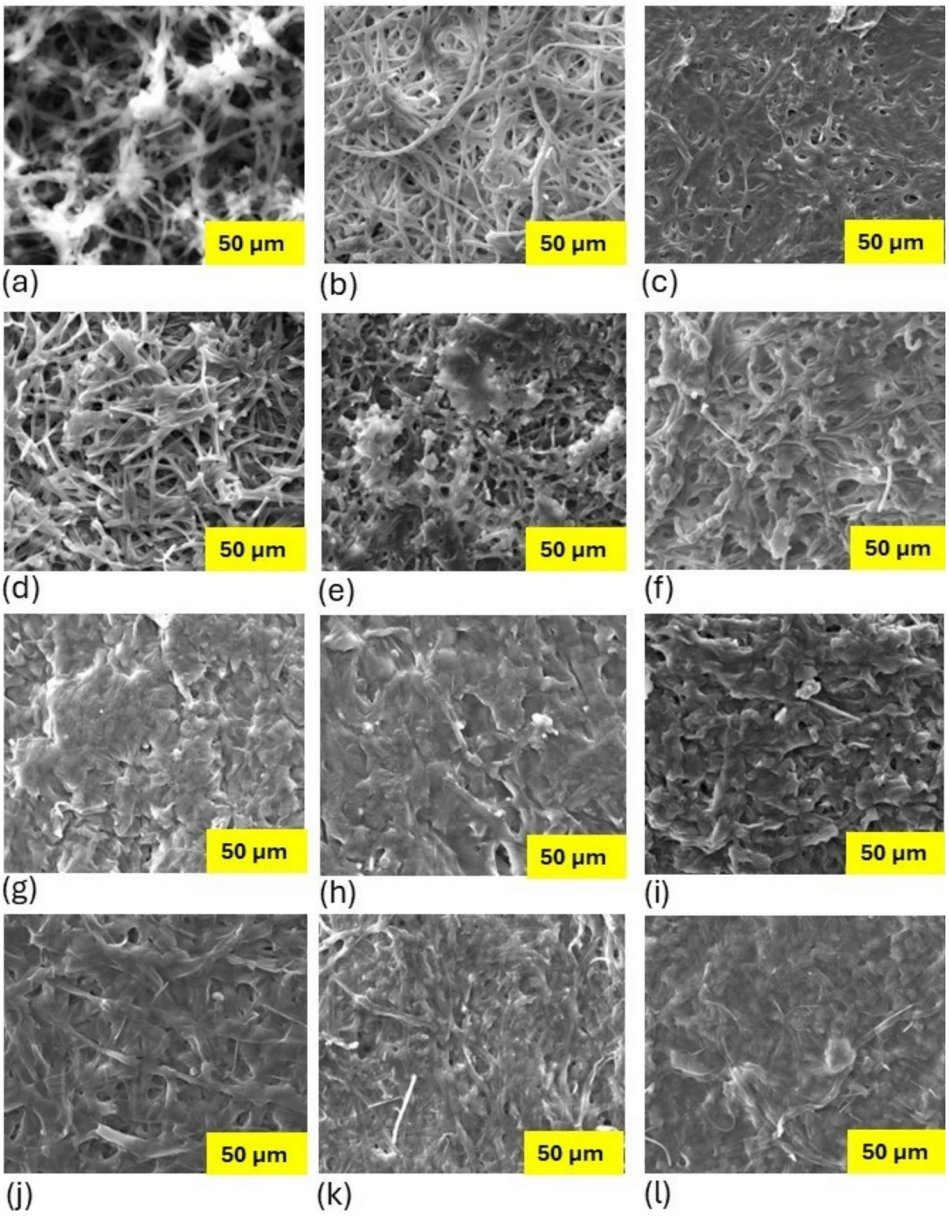

FIGURE 7 Morphological characteristics of pure mycelium materials (PMM) shown by scanning electron microscopy (SEM) for: (a) *T. versicolor* cultivated in PDB; (b) *T. versicolor* cultivated in MEB; (c) *T. versicolor* cultivated in agri- processing water-based feedstock; (d) *I. lacteus* cultivated in PDB; (e) *I. lacteus* in MEB; (f) *I. lacteus* cultivated in agri-processing water-based feedstock; (g) 10% v/v glycerol-treated *I. lacteus* cultivated in PDB; (h) 30% v/v glycerol-treated *I. lacteus* cultivated in PDB; (i) 10% v/v glycerol-treated *I. lacteus* cultivated in MEB; (j) 30% v/v glycerol-treated *I. lacteus* cultivated in MEB; (k) 10% v/v glycerol-treated *I. lacteus* cultivated in agri-processing water-based feedstock; and (l) 30% v/v glycerol-treated *I. lacteus* cultivated in agri-processing water-based feedstock.

hydrogen bonds between glycerol and cell wall components, as discussed above. Consequently, this disrupted and weakened the intramolecular hydrogen bonding among cell wall components, resulting in a more flexible and softer appearance (Peltzer et al., 2018; Suppakul et al., 2013). This soft and smooth appearance offers the benefit of producing uniform packaging materials, enhancing consumer appeal and acceptance.

Density of PMMs

The density of PMMs was assessed using a densimeter, and the results are summarized in Table 2. It was found that PMMs cultivated on agri-processing water exhibited higher densities compared to those grown on synthetic media. For *T. versicolor*-based PMMs, the highest density recorded was 0.831 ± 0.02 g/cm^3 for samples grown on 10% non-autoclaved agri-processing water. In contrast, PMMs derived from synthetic media displayed lower densities, with PDB-based PMM showing a density of 0.603 ± 0.08 g/cm^3, and MEB-based PMM exhibiting the lowest density at 0.428 ± 0.08 g/cm^3.

A similar trend was observed for PMMs produced from *I. lacteus*, where agri-processing water-based PMMs had higher densities. Specifically, a density of 0.757 ± 0.05 g/cm^3 was measured for PMMs grown in 10% autoclaved agri-processing water, while 0.825 ± 0.03 g/cm^3 was recorded for PMMs from 10% non-autoclaved agri-processing water. Morphological analysis via SEM (Section 3.4) supported these findings, revealing that PMMs from synthetic media exhibited entangled,

TABLE 2 Densities of pure mycelium materials (PMM) grown on various feedstocks.

PMM type	Without glycerol treatment	With glycerol treatment	
		10% glycerol	30% glycerol
T. versicolor in PDB	0.603 ± 0.08		
T. versicolor in MEB	0.428 ± 0.09		
T. versicolor in agri-processing water	0.752 ± 0.05		
I. lacteus in PDB	0.419 ± 0.04	1.068 ± 0.32	1.110 ± 0.03
I. lacteus in MEB	0.186 ± 0.07	0.835 ± 0.19	0.905 ± 0.09
I. lacteus in agri-processing water	0.757 ± 0.05	0.877 ± 0.13	0.940 ± 0.04

Note: Density is reported in g/cm^3.

tubelike structures, whereas those from agri-processing water showed tightly packed, less porous, and flatter surface morphologies, accounting for their higher densities. Similar results were reported by Antinori et al. (2020), who obtained denser materials when grown on lignin-based substrates compared to those grown on synthetic media such as PDB.

Treatment with glycerol led to a further increase in the density of the synthesized PMMs, with a general trend of higher density across all treated samples. In particular, PMMs grown in PDB using *I. lacteus* exhibited a 154% and 165% increase in density when treated with 10% v/v and 30% v/v glycerol, respectively, compared to untreated samples. Similarly, glycerol treatment significantly increased the density of *I. lacteus* PMMs grown in MEB, with increments of 345% and 386% for 10% v/v and 30% v/v glycerol, respectively. However, the increase was less pronounced for PMMs derived from agri-processing water, where density rose by 15.8% with 10% v/v glycerol and 24.2% with 30% v/v glycerol.

A similar trend was reported by Errico et al. (2024), where PMM density increased from 1.153 to 1.806 g/cm^3 as the concentration of glutaraldehyde (plasticizer) rose from 0.01% to 1%. Thus, the density increase with higher glycerol concentrations observed in the present study may be attributed to the presence of glycerol, which has a density of 1.26 g/cm^3, thereby contributing to the overall increase in PMM density (Moore et al., 2006). These obtained densities (<1.0 g/cm^3) for PMMs are comparable to LDPE (0.910–0.925 g/cm^3). Such low densities may offer a weight saving advantage making them a suitable substitute for SUPs such as LDPE in food packaging (Jordan et al., 2016).

Mechanical behavior of PMMs

The viscoelastic behavior of PMMs in the presence of temperature and frequency was analyzed using DMA, focusing on the measurements of E′, E″, and Tan δ (Figure 8). It was observed that the synthesized PMMs exhibited a brittle behavior and often fractured during clamping. This necessitated their post-processing treatment to enhance their flexibility before DMA analysis could be performed. In the present study, this enhancement was achieved through post-processing the PMMs in 10% and 30% v/v glycerol, a plasticizer.

E′ represents the ability of a material to store energy in an elastic manner (Antinori et al., 2020). This property highlights how a material's stiffness and ability to bear loads change with temperature (Gudayu et al., 2021). Assessing these variations is essential for determining whether a material retains adequate rigidity at room temperature, which is particularly needed for specific packaging applications, for example, food containers. If the material is thermal sensitive, modifications such as treatment with plasticizers or cross-linking agents may be required to improve its structural stability and functionality. This phenomenon was observed for PMMs when evaluated directly upon drying, and therefore, in this study, the synthesized PMMs were treated with glycerol to enhance the load endurance ability.

An increment in E′ of PDB-based PMM from *I. lacteus* treated with 10% v/v glycerol was observed from approximately 33.0 MPa at room temperature (25 ± °C) to 41.2 MPa at 47°C. This surge in stiffness with rising temperature may result from the alignment of mycelium fibers along the applied stress, which helped suppress the mechanical vibrations (Bustillos et al., 2020). Beyond 47°C, the PMM behavior shifted to a leathery region as observed by the reduction in the E′ to approximately 18.0 MPa at 80°C. In this region, a material remains tough but flexible (Karunarathne et al., 2024). On treatment with 30% v/v glycerol, an 87.2% reduction in E′ at room temperature was observed. This reduction led to a decrease in stiffness, which could be attributed to the enhanced plasticizing effect of the 30% v/v glycerol. The incorporation of glycerol facilitates

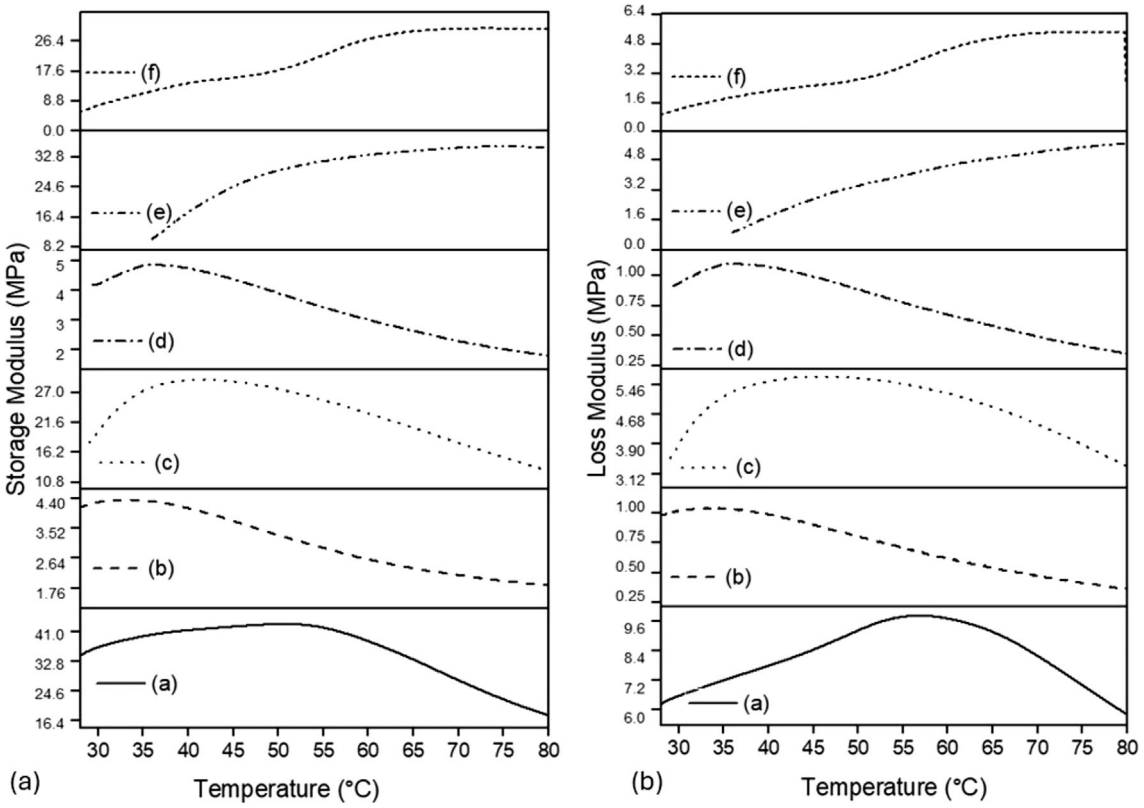

FIGURE 8 **Dynamic mechanical analysis (DMA) of glycerol-treated pure mycelium materials (PMM) synthesized by *I. lacteus* on various feedstocks.** Figures (a) and (b) show the storage modulus and loss modulus, respectively, of samples: (a) 10% v/v glycerol-treated PDB-based PMM; (b) 30% v/v glycerol-treated PDB-based PMM; (c) 10% v/v glycerol-treated MEB-based PMM; (d) 30% v/v glycerol-treated MEB-based PMM; (e) 10% v/v glycerol-treated 10% agri-processing water-based PMM; and (f) 30% v/v glycerol-treated 10% agri-processing water-based PMM.

hydrogen bonding with the components of the fungal cell wall, creating more intermolecular space and thus increasing flexibility (Appels et al., 2020). This improved flexibility likely contributed to the observed decrease in stiffness and E′ (Sahoo et al., 2014). These findings align with those reported by Appels et al. (2020), who investigated glycerol treatment levels ranging from 0% to 32% in *Schizophyllum commune*-based PMMs synthesized on synthetic minimal medium. It was reported that the mechanical properties such as Young's modulus and tensile strength of the synthesized PMMs decreased with increasing glycerol concentrations.

The MEB-based PMMs exhibited a similar trend to the PDB-based PMMs. At room temperature, a E′ of approximately 18.0 MPa was recorded, which decreased to 4.0 MPa (30% v/v glycerol treatment). Both the 30% v/v glycerol-treated PDB and MEB-based PMMs transitioned into the leathery region at lower temperatures compared to those treated with 10% v/v glycerol treatment. This suggested that the addition of a plasticizer made the materials more malleable at lower temperatures, potentially compromising the stability of PMMs for storing and carrying products when higher temperatures, such as 35°C, are used. Therefore, a 10% v/v glycerol concentration was found to be more effective than 30% v/v glycerol in maintaining flexibility and higher E′, which is needed for practical applications in packaging.

The treatment of 10% agri-processing water-based PMMs from *I. lacteus* with 10% v/v glycerol increased the E′ to 34.0 MPa at 65°C in comparison to 30% v/v glycerol-treated PMM with E′ of 29.0 MPa at approximately 62°C. Beyond this temperature, the PMMs transitioned to leathery region. This shift to leather region at higher temperature in comparison to synthetic media-based PMMs suggested that these materials could hold higher loads at higher temperatures such as 60°C making them suitable for wide range of packaging applications.

E″ represents the energy lost as heat when a material is heated, which is primarily due to the internal friction within the material. Energy dissipation is influenced by several factors, including polymer structure, interactions between additives and the polymer matrix, and matrix cracking. Therefore, higher energy dissipation can

indicate a weaker polymer matrix interface, thereby impacting the structural integrity of the materials (Gudayu et al., 2021). An increase in glycerol concentration from 10% to 30% v/v resulted in a decrease in E″ value across all samples. Glycerol functions as a plasticizer, enhancing ductility and reducing the internal resistance, which in turn lowers energy dissipation. The E″ value of PDB-based PMMs was higher in materials treated with 10% v/v glycerol, reaching a peak of approximately 9.8 MPa at 57°C, whereas for 30% v/v glycerol-treated materials, it declined to 1.05 MPa at 36°C. A similar pattern was observed in MEB-based PMMs, where E″ decreased from 5.6 MPa at 50°C to 1.08 MPa at 36°C for 10% and 30% v/v glycerol-treated PMMs, respectively. While this reduction in E″ can be beneficial, the significant energy dissipation at such low temperatures is undesirable for certain packaging applications. It compromises structural integrity, making the material unsuitable, for example, to carry hot meals or for use in regions with high ambient temperature conditions, such as 40°C. Glycerol treatment also lowered the E″ for 10% agri-processing water-based PMMs. A decrease from 5.0 MPa at 65°C to 4.8 MPa at 60°C was observed for PMMs treated with 10% v/v and 30% v/v glycerol, respectively. The minimal difference in E″ between these treatments suggested that both variants could be suitable for a various packaging applications.

Tan δ reflects a material's capacity to dissipate energy as heat through its viscous component (loss modulus—E″) relative to its ability to store energy through its elastic component (storage modulus—E′) (Bustillos et al., 2020). In principle, it refers to the molecular mobility of materials, that is, a larger estimation of tan δ is described by a high molecular movement among the interfaces. In the present study, it was observed that increasing the glycerol concentration resulted in higher tan δ values as mentioned in Table 3. This behavior correlates with the reduction in E′, indicating a transition towards a more viscoelastic (less elastic) behavior, which led to elevated tan δ values. A similar observation was reported by Antinori et al. (2020) where a decrease in tan δ from 0.347 ± 0.013 to 0.219 ± 0.007 for glucose media-based *G. lucidum* PMMs-induced brittleness. The increased glycerol concentrations appear to enhance the ductility of the PMM, resulting in a significant reduction in the storage modulus, while the loss modulus decreases at a slower rate, thereby increasing tan δ. This trend suggested that glycerol acted as an effective plasticizer for the modification of PMMs, improving the damping properties of the PMM while simultaneously reducing its stiffness and elasticity. In contrast, neat LDPE displayed a tan δ of 0.25, while neat PLA reached a maximum tan δ of 1.3. The PMMs in this study exhibited tan δ values comparable to LDPE, indicating lower viscoelastic behavior than PLA. This characteristic makes PMMs well-suited for producing packaging materials, such as LDPE-based food wraps (Kumar et al., 2024; Suresha et al., 2022). The findings from this work on post-processing can enhance the understanding on how plasticizers influence PMMs, enabling the optimization of desirable characteristics to suit specific packaging applications.

TABLE 3 Data of dynamic mechanical analysis (DMA) for glycerol-treated pure mycelium materials (PMM).

PMM type	Glycerol concentration% (v/v)	Tan δ
I. lacteus in PDB	10%	0.21 ± 0.05
	30%	0.24 ± 0.005
I. lacteus in MEB	10%	0.21 ± 0.04
	30%	0.22 ± 0.004
I. lacteus in 10% agri-processing water	10%	0.12 ± 0.04
	30%	0.14 ± 0.01

CONCLUSIONS

In this study, PMMs were developed which are self-growing and fibrous bio-based materials with a potentially benign carbon footprint. It was demonstrated that the variations in feedstock composition significantly altered the fungal cell wall composition and hyphal structure, ultimately affecting the final PMM properties.

PMMs were successfully cultivated on lignocellulosic-rich agri-processing water as a sole feedstock without any supplementation with pure and expensive nutrients, which could provide significant cost savings in the overall PMM production cost. Mycelium exhibited a faster growth on synthetic media due to their simpler composition compared to the lignocellulosic-rich wastewater stream. However, PMMs derived from agri- processing water exhibited superior densities and mechanical behavior that were suitable for packaging applications. These findings provided a correlation between feedstock properties and characteristics of the resultant PMM and suggested that PMMs can be engineered with tunable properties through modifications in the growth medium conditions. Since the agri-processing water contained some growth inhibitors, it would be important to evaluate these byproducts and determine if and how the agri-processing water might be further treated to develop an optimal feedstock for high PMM production.

As such, PMMs exhibited a brittle nature upon drying, a challenge that needed to be overcome for their

development as packaging materials. To address this issue, PMMs were post-processed with two glycerol concentrations, 10% v/v and 30% v/v, to examine their mechano-physical behavior with a rise in glycerol concentration. The compositional analysis of synthesized PMMs by FTIR revealed the presence of fungal cell wall components such as proteins, chitin, and other polysaccharides. The interaction between glycerol and cell wall components in the post-processed PMMs was also confirmed using this technique. Such interaction modified the surface of PMMs turning them smooth from previously entangled form in non-treated PMM due to the penetration of glycerol into the porous structures, as observed through the SEM analysis. This smoother and softer appearance enhanced the potential for developing uniform-surfaced packaging materials, improving both end user appeal and acceptance.

Thermal analysis of PMMs demonstrated a thermal stability comparable to LDPE with major degradation in the temperature range 200–380°C resulting from the disintegration of structural components such as polysaccharides, proteins, and chitin within the mycelium cell wall. The comparable T_m of produced PMMs to LDPE and PLA showed their amenability for manufacturing techniques such as injection molding or extrusion, to enable the development of desirable PMM-based packaging products. Furthermore, agri-processing water-based PMMs exhibited a higher density compared to those derived from synthetic media, and glycerol treatment led to a further enhancement in these densities. The post-processed PMMs from both synthetic and agri-processing water displayed densities comparable to LDPE, further proving their suitability as SUP packaging alternatives. Higher glycerol concentrations (30% v/v) also enhanced their malleability, resulting in a decrease in both E′ and E″ and indicating a more flexible behavior. However, the apparent shift in E′ and E″ at lower temperatures (35°C) for 30% v/v glycerol-treated PMMs may compromise their structural integrity, making them less suitable for applications dealing with hot products or for regions with higher temperatures. Nevertheless, glycerol effectively acted as a plasticizer and imparted ductility to PMMs.

Overall, by integrating agri-processing water-based feedstock into PMM production, this study demonstrated a viable pathway for converting low-value but nutrient-rich agri-processing water into commercially relevant biomaterials. Furthermore, the mechano-physical properties of PMMs, particularly with post-processing treatments, provided further support to their development as suitable packaging alternatives. As the next step, mass scale production of PMMs with target mechano-physical properties will be developed using bioreactors to advance the PMM technology for diverse packaging applications.

AUTHOR CONTRIBUTIONS

Malvika Sharma: Conceptualization; methodology; data curation; formal analysis; investigation; writing—original draft; writing—review and editing. **Livia Fleischmann**: Investigation. **Maxwell McInnis**: Investigation. **Arturo Rodriguez-Uribe**: Investigation; writing—review and editing. **Manjusri Misra**: Resources; writing—review and editing. **Loong-Tak Lim**: Conceptualization; supervision; funding acquisition; writing—review and editing. **Guneet Kaur**: Conceptualization; funding acquisition; project administration; data curation; resources; supervision; writing—review and editing.

ACKNOWLEDGEMENTS

This research is funded by the Ontario Ministry of Agriculture, Food, and Agribusiness (OMAFA), previously Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), through the Ontario Agri-Food Innovation Alliance, Grant number: UGT1-2024-102362.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Guneet Kaur https://orcid.org/0000-0003-2293-9708

REFERENCES

Agrawal, P. K., Bhatt, C. S., & Viswanathan, L. (1983). Effect of some metabolic inhibitors on citric acid production by *Aspergillus niger*. *Enzyme and Microbial Technology*, *5*(5), 373–376. https://doi.org/10.1016/0141-0229(83)90011-X

Antinori, M. E., Ceseracciu, L., Mancini, G., Heredia-Guerrero, J. A., & Athanassiou, A. (2020). Fine-tuning of physicochemical properties and growth dynamics of mycelium-based materials. *ACS Applied Bio Materials*, *3*(2), 1044–1051. https://doi.org/10.1021/acsabm.9b01031

Antinori, M. E., Contardi, M., Suarato, G., Armirotti, A., Bertorelli, R., Mancini, G., Debellis, D., & Athanassiou, A. (2021). Advanced mycelium materials as potential self-growing biomedical scaffolds. *Scientific Reports*, *11*(1), 12630. https://doi.org/10.1038/s41598-021-91572-x

Appels, F. V. W., van den Brandhof, J. G., Dijksterhuis, J., de Kort, G. W., & Wösten, H. A. B. (2020). Fungal mycelium classified in different material families based on glycerol treatment. *Communications Biology*, *3*(1), 334. https://doi.org/10.1038/s42003-020-1064-4

Basil, M., Anirudh, M. K., Lal, A. N., Harikrishnan, M. P., Kundu, P., & Kothakota, A. (2023). Development and

characterization of microfiber incorporated with industrial biopolymer composite based biodegradable cutlery: An alternative to single use plastic. *Industrial Crops and Products*, *205*, 117526. https://doi.org/10.1016/j.indcrop.2023.117526

Ben, Z. Y., Samsudin, H., & Yhaya, M. F. (2022). Glycerol: Its properties, polymer synthesis, and applications in starch based films. *European Polymer Journal*, *175*, 111377. https://doi.org/10.1016/j.eurpolymj.2022.111377

Bustillos, J., Loganathan, A., Agrawal, R., Gonzalez, B. A., Perez, M. G., Ramaswamy, S., Boesl, B., & Agarwal, A. (2020). Uncovering the mechanical, thermal, and chemical characteristics of biodegradable mushroom leather with intrinsic antifungal and antibacterial properties. *ACS Applied Bio Materials*, *3*(5), 3145–3156. https://doi.org/10.1021/acsabm.0c00164

Chen, H., Liang, X., Li, S., Wang, Z., Li, K., Feng, T., Ni, Z., & Zhu, D. (2022). Study on mechanism of wheat straw lignin degradation by lignin peroxidase from *Irpex lacteus*

Cruz, R. M. S., Krauter, V., Krauter, S., Agriopoulou, S., Weinrich, R., Herbes, C., Scholten, P. B. V., Uysal-Unalan, I., Sogut, E., Kopacic, S., Lahti, J., Rutkaite, R., & Varzakas, T. (2022). Bioplastics for food packaging: Environmental impact, trends and regulatory aspects. *Food*, *11*(19), 3087. https://doi.org/10.3390/foods11193087

Duan, Z., Shen, R., Liu, B., Yao, M., & Jia, R. (2018). Comprehensive investigation of a dye-decolorizing peroxidase and a manganese peroxidase from *Irpex lacteus* F17, a lignin-degrading basidiomycete. *AMB Express*, *8*(1), 119. https://doi.org/10.1186/s13568-018-0648-6

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

El-Zaher, E., Mahmoud, Y. A. G., & Aly, M. M. (2011). Effect of different concentrations of phenol on growth of some fungi isolated from contaminated soil. *African Journal of Biotechnology*, *10*(8), 1384–1392.

Environment and Climate Change Canada. (2022). Single-use Plastics Prohibition Regulations: Overview [Internet]. www.canada.ca. Government of Canada, Available from: https://www.canada.ca/en/environment-climate-change/services/managing-reducing-waste/reduce-plastic-waste/single-use-plastic-overview.html

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

Farinha, I., Duarte, P., Pimentel, A., Plotnikova, E., Chagas, B., Mafra, L., Grandfils, C., Freitas, F., Fortunato, E., & Reis, M. A. M. (2015). Chitin–glucan complex production by *Komagataella pastoris*: Downstream optimization and product characterization. *Carbohydrate Polymers*, *130*, 455–464. https://doi.org/10.1016/j.carbpol.2015.05.034

Fernández-Fueyo, E., Ruiz-Dueñas, F. J., López-Lucendo, M. F., Pérez-Boada, M., Rencoret, J., Gutiérrez, A., Pisabarro, A. G., Ramírez, L., & Martínez, A. T. (2016). A secretomic view of woody and nonwoody lignocellulose degradation by *Pleurotus ostreatus*. *Biotechnology for Biofuels*, *9*, 1–18. https://doi.org/10.1186/s13068-016-0462-9

Gao, C., Stading, M., Wellner, N., Parker, M. L., Noel, T. R., Mills, E. N. C., & Belton, P. S. (2006). Plasticization of a protein-based film by glycerol: A spectroscopic, mechanical, and thermal study. *Journal of Agricultural and Food Chemistry*, *54*(13), 4611–4616. https://doi.org/10.1021/jf060611w

Gudayu, A. D., Steuernagel, L., Meiners, D., & Gideon, R. (2021). Characterization of the dynamic mechanical properties of sisal fiber reinforced PET composites; effect of fiber loading and fiber surface modification. *Polymers and Polymer Composites*, *29*(9_suppl), S719–S728. https://doi.org/10.1177/09673911211023032

Guerrero, J. A. H., Benítez, J. J., Porras-Vázquez, J. M., Tedeschi, G., Morales, Y., Fernández-Ortuño, D., Athanassiou, A., & Guzman-Puyol, S. (2023). Plasticized, greaseproof chitin bioplastics with high transparency and biodegradability. *Food Hydrocolloids*, *145*, 109072. https://doi.org/10.1016/j.foodhyd.2023.109072

Guo, Z., Ge, X., Yang, L., Gou, Q., Han, L., & Yu, Q. (2021). Utilization of watermelon peel as a pectin source and the effect of ultrasound treatment on pectin film properties. *LWT*, *147*, 111569. https://doi.org/10.1016/j.lwt.2021.111569

Haneef, M., Ceseracciu, L., Canale, C., Bayer, I. S., Heredia-Guerrero, J. A., & Athanassiou, A. (2017). Advanced materials from fungal mycelium: Fabrication and tuning of physical properties. *Scientific Reports*, *7*(1), 41292. https://doi.org/10.1038/srep41292

Husárová, L., Pekařová, S., Stloukal, P., Kucharzcyk, P., Verney, V., Commereuc, S., Ramone, A., & Koutny, M. (2014). Identification of important abiotic and biotic factors in the biodegradation of poly (l-lactic acid). *International Journal of Biological Macromolecules*, *71*, 155–162. https://doi.org/10.1016/j.ijbiomac.2014.04.050

Iqbal, H. M. N., Asgher, M., & Bhatti, H. N. (2011). Optimization of physical and nutritional factors for synthesis of lignin degrading enzymes by a novel strain of *Trametes versicolor*. *BioResources*, *6*(2), 1273–1287. https://doi.org/10.15376/biores.6.2.1273-1287

Jang, M., Kong, B., Jeong, Y., Lee, C. H., & Nah, J. (2004). Physicochemical characterization of α-chitin, β-chitin, and γ-chitin separated from natural resources. *Journal of Polymer Science Part A: Polymer Chemistry*, *42*(14), 3423–3432. https://doi.org/10.1002/pola.20176

Jordan, J. L., Casem, D. T., Bradley, J. M., Dwivedi, A. K., Brown, E. N., & Jordan, C. W. (2016). Mechanical properties of low density polyethylene. *Journal of Dynamic Behavior of Materials*, *2*(4), 411–420. https://doi.org/10.1007/s40870-016-0076-0

Joyline, M., & Aruna, K. (2019). Production and characterization of polyhydroxyalkanoates (PHA) by *Bacillus megaterium* strain JHA using inexpensive agro-industrial wastes. *International Journal of Recent Scientific Research*, *10*(7), 33359–33374.

Karunarathne, A., Nabiyeva, G., Rasmussen, C. J., Alkhoury, K., Assem, N., Bauer, J., Chester, S. A., Khalizov, A. F., & Gor, G. Y. (2024). Effects of humidity on mycelium-based leather. *ACS Applied Bio Materials*, *7*(10), 6441–6450. https://doi.org/10.1021/acsabm.4c00586

Khamrai, M., Banerjee, S. L., & Kundu, P. P. (2018). A sustainable production method of mycelium biomass using an isolated

fungal strain *Phanerochaete chrysosporium* (accession no: KY593186): Its exploitation in wound healing patch formation. *Biocatalysis and Agricultural Biotechnology*, *16*, 548–557. https://doi.org/10.1016/j.bcab.2018.09.013

Kumar, A., & Chandra, R. (2020). Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. *Heliyon*, *6*(2), e03170. https://doi.org/10.1016/j.heliyon.2020.e03170

Kumar, J., Kumar, A., Maurya, A. K., Gupta, H. S., Singh, S. P., & Sharma, C. (2024). Utilization of *Ananas comosus* crown residue husk as a sustainable strength additive for EPR/LDPE blend composites. *ACS Omega*, *9*(2), 2740–2751. https://doi.org/10.1021/acsomega.3c07697

Moore, G. R. P., Maria Martelli, S., Gandolfo, C., José do Amaral Sobral, P., & Borges Laurindo, J. (2006). Influence of the glycerol concentration on some physical properties of feather keratin films. *Food Hydrocolloids*, *20*(7), 975–982. https://doi.org/10.1016/j.foodhyd.2005.11.001

Nasir, N. N., & Othman, S. A. (2020). Application of bioplastic packaging in industry. *Journal of Advanced Research in Materials Science*, *74*(1), 19–28. https://doi.org/10.37934/arms.74.1.1928

Peeters, E., Salueña Martin, J., & Vandelook, S. (2023). Growing sustainable materials from filamentous fungi. http://portlandpress.com/biochemist/article-pdf/45/3/8/947523/bio_2023_120.pdf

Peltzer, M. A., Salvay, A. G., Delgado, J. F., de la Osa, O., & Wagner, J. R. (2018). Use of residual yeast cell wall for new biobased materials production: Effect of plasticization on film properties. *Food and Bioprocess Technology*, *11*(11), 1995–2007. https://doi.org/10.1007/s11947-018-2156-8

Pilafidis, S., Tsouko, E., Sougleri, G., Diamantopoulou, P., Gkatzionis, K., Ioannou, Z., & Sarris, D. (2024). Submerged cultivation of selected macro-fungi to produce mycelia rich in β-glucans and other bioactive compounds, valorizing side streams of the food industry. *Carbon Resources Conversion*, *7*(2), 100198. https://doi.org/10.1016/j.crcon.2023.09.002

Qin, X., Su, X., Luo, H., Ma, R., Yao, B., & Ma, F. (2018). Deciphering lignocellulose deconstruction by the white rot fungus *Irpex lacteus* based on genomic and transcriptomic analyses. *Biotechnology for Biofuels*, *11*(1), 58. https://doi.org/10.1186/s13068-018-1060-9

Rafiee, K., Schritt, H., Pleissner, D., Kaur, G., & Brar, S. K. (2021). Biodegradable green composites: It's never too late to mend. *Current Opinion in Green and Sustainable Chemistry*, *30*, 100482. https://doi.org/10.1016/j.cogsc.2021.100482

Rathinamoorthy, R., Bharathi, T. S., Snehaa, M., & Swetha, C. (2023). Structural and chemical characterization of mycelium sheets developed from *Penicillium camemberti*. *Journal of Polymers and the Environment*, *31*(12), 5152–5165. https://doi.org/10.1007/s10924-023-02941-8

Sahoo, S., Misra, M., & Mohanty, A. K. (2014). Biocomposites from switchgrass and lignin hybrid and poly(butylene succinate) bioplastic: Studies on reactive compatibilization and performance evaluation. *Macromolecular Materials and Engineering*, *299*(2), 178–189. https://doi.org/10.1002/mame.201300038

Saini, R., Kaur, G., & Brar, S. K. (2024). Textile residue-based mycelium biocomposites from *Pleurotus ostreatus*. *Mycology*, *15*(4), 683–689. https://doi.org/10.1080/21501203.2023.2278308

Sharma, M., Beniwal, P., & Toor, A. P. (2022). The effect of rice straw derived microfibrillated cellulose as a reinforcing agent in starch/polyvinyl alcohol/polyethylene glycol biocompatible films. *Materials Chemistry and Physics*, *291*, 126652. https://doi.org/10.1016/j.matchemphys.2022.126652

Shin, J., Liu, X., Chikthimmah, N., & Lee, Y. S. (2016). Polymer surface modification using UV treatment for attachment of natamycin and the potential applications for conventional food cling wrap (LDPE). *Applied Surface Science*, *386*, 276–284. https://doi.org/10.1016/j.apsusc.2016.05.158

Smiderle, F. R., Olsen, L. M., Ruthes, A. C., Czelusniak, P. A., Santana-Filho, A. P., Sassaki, G. L., Gorin, P. A. J., & Iacomini, M. (2012). Exopolysaccharides, proteins and lipids in *Pleurotus pulmonarius* submerged culture using different carbon sources. *Carbohydrate Polymers*, *87*(1), 368–376. https://doi.org/10.1016/j.carbpol.2011.07.063

Sorolla-Rosario, D., Llorca-Porcel, J., Pérez-Martínez, M., Lozano-Castelló, D., & Bueno-López, A. (2022). Study of microplastics with semicrystalline and amorphous structure identification by TGA and DSC. *Journal of Environmental Chemical Engineering*, *10*(1), 106886. https://doi.org/10.1016/j.jece.2021.106886

Stark, N. M., & Matuana, L. M. (2021). Trends in sustainable biobased packaging materials: A mini review. *Materials Today Sustainability*, *15*, 100084. https://doi.org/10.1016/j.mtsust.2021.100084

Suppakul, P., Chalernsook, B., Ratisuthawat, B., Prapasitthi, S., & Munchukangwan, N. (2013). Empirical modeling of moisture sorption characteristics and mechanical and barrier properties of cassava flour film and their relation to plasticizing–antiplasticizing effects. *LWT - Food Science and Technology*, *50*(1), 290–297. https://doi.org/10.1016/j.lwt.2012.05.013

Suresha, B., Giraddi, V. V., Anand, A., & Somashekar, H. M. (2022). Dynamic mechanical analysis of 3D printed carbon fiber reinforced polylactic acid composites. *Materials Today Proceedings*, *59*, 794–799. https://doi.org/10.1016/j.matpr.2022.01.035

Sydor, M., Bonenberg, A., Doczekalska, B., & Cofta, G. (2021). Mycelium-based composites in art, architecture, and interior design: A review. *Polymers*, *14*(1), 145. https://doi.org/10.3390/polym14010145

Türkkan, M., & Erper, I. (2014). Evaluation of antifungal activity of sodium salts against onion basal rot caused by *Fusarium oxysporum* f. sp. cepae. *Plant Protection Science*, *50*(1), 19–25. https://doi.org/10.17221/9/2013-PPS

Vandelook, S., Elsacker, E., Van Wylick, A., De Laet, L., & Peeters, E. (2021). Current state and future prospects of pure mycelium materials. *Fungal Biology and Biotechnology*, *8*, 1–10. https://doi.org/10.1186/s40694-021-00128-1

Veena, S. S., & Pandey, M. (2012). Physiological and cultivation requirements of *Trametes versicolor*, a medicinal mushroom to diversify Indian mushroom industry. *Indian Journal of Agricultural Sciences*, *82*(8), 672–675. https://doi.org/10.56093/ijas.v82i8.23047

Wan, C., & Li, Y. (2012). Fungal pretreatment of lignocellulosic biomass. *Biotechnology Advances*, *30*(6), 1447–1457. https://doi.org/10.1016/j.biotechadv.2012.03.003

Wang, J., Tavakoli, J., & Tang, Y. (2019). Bacterial cellulose production, properties and applications with different culture methods–A review. *Carbohydrate Polymers*, *219*, 63–76. https://doi.org/10.1016/j.carbpol.2019.05.008

Welle, F. (2011). Twenty years of PET bottle to bottle recycling—An overview. *Resources, Conservation and Recycling*, *55*(11), 865–875. https://doi.org/10.1016/j.resconrec.2011.04.009

Wijayarathna, E. R. K. B., Mohammadkhani, G., Soufiani, A. M., Adolfsson, K. H., Ferreira, J. A., Hakkarainen, M., Berglund, L., Heinmaa, I., Root, A., & Zamani, A. (2022). Fungal textile alternatives from bread waste with leather-like properties. *Resources, Conservation and Recycling*, *179*, 106041. https://doi.org/10.1016/j.resconrec.2021.106041

Xia, S., Shen, S., Song, J., Li, K., Qin, X., Jiang, X., Xue, C., & Xue, Y. (2023). Physicochemical and structural properties of meat analogues from yeast and soy protein prepared via high-moisture extrusion. *Food Chemistry*, *402*, 134265. https://doi.org/10.1016/j.foodchem.2022.134265

Xiao, Q., Ma, F., Li, Y., Yu, H., Li, C., & Zhang, X. (2017). Differential proteomic profiles of *Pleurotus ostreatus* in response to lignocellulosic components provide insights into divergent adaptive mechanisms. *Frontiers in Microbiology*, *8*, 480. https://doi.org/10.3389/fmicb.2017.00480

Yu, M., Zheng, Y., & Tian, J. (2020). Study on the biodegradability of modified starch/polylactic acid (PLA) composite materials. *RSC Advances*, *10*(44), 26298–26307. https://doi.org/10.1039/D0RA00274G

How to cite this article: Sharma, M., Fleischmann, L., McInnis, M., Rodriguez-Uribe, A., Misra, M., Lim, L.-T., & Kaur, G. (2025). Pure mycelium materials production from agri-processing water: Effects of feedstock composition on material properties for packaging applications. *Water Environment Research*, *97*(5), e70089. https://doi.org/10.1002/wer.70089