Biofabrication of Composites Based on Mycelium and Agave Fiber (*Agave tequilana* Weber var. azul)

Alexandra Miguel Guevara-Castillo,[a] José Antonio Gutiérrez Ortega,[a] Alma Rosa Villalobos Arámbula,[b] María del Pilar Zamora Tavares,[c] and María Guadalupe Lomelí-Ramírez,[a,*]

Expanded polystyrene (EPS), widely used in plastic packaging, poses recycling challenges and contributes to environmental pollution due to its persistence. As an alternative, biopackaging materials were developed using mycelium from *Ganoderma lucidum* and agave bagasse (*Agave tequilana* Weber var. azul), a common agro-industrial residue in Jalisco, Mexico. The resulting materials showed a uniform, lightweight structure. SEM analysis confirmed full colonization of agave fibers by mycelium, indicating good cohesion. FTIR spectroscopy suggested intermolecular interactions between the fungal matrix and the lignocellulosic substrate. The material also exhibited hydrophobic behavior, as evidenced by its high water contact angle, indicating a surface with low wettability. In fire tests, the biopackaging burned slowly, emitted white smoke, and exhibited self-extinguishing behavior. Mechanical testing showed that the composites reached maximum force values in the same order of magnitude as EPS. Although flexural strength was slightly lower, the material tolerated greater deformation under comparable loads, indicating improved strain accommodation and resistance to abrupt fracture. Using the LIDS (Life Cycle Design Strategy Wheel), these materials were compared to conventional plastics, demonstrating advantages such as reduced reliance on petroleum-based polymers, valorization of agave waste, and alignment with circular production strategies in the tequila sector.

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*Contact information: a: Departamento de Madera, Celulosa y Papel, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Km 15.5 Carretera Guadalajara-Nogales, Las Agujas, 45020 Zapopan, Jalisco, México; b: Departamento de Biología Celular y Molecular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Apdo. Postal 39-82, Zapopan, Jal. 45110, México; c: Departamento de Botánica y Zoología Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Km 15.5 Carretera Guadalajara-Nogales, Las Agujas, 45020 Zapopan, Jalisco, México; *Corresponding author: maria.lramirez@academicos.udg.mx*

INTRODUCTION

Plastic packaging, widely used across various applications, presents a major environmental challenge due to its single-use nature and recycling complexity. The addition of blends and additives during manufacturing further complicates end-of-life processing, and its fossil origin from petroleum limits biodegradability. Polystyrene, commonly used for food packaging and protective materials in different industries, offers advantages such as rigidity, light weight, and low cost (Carreño-Farfán *et al*. 2024). However, its fossil origin and slow degradation make it a persistent pollutant in terrestrial and marine ecosystems. Therefore, the development of eco-friendly packaging alternatives is necessary. Society increasingly demands sustainable, environmentally friendly, and recyclable materials, while the circular economy framework promotes renewable solutions for conventional material use.

Biofabrication, which integrates biology, biotechnology, and engineering, has emerged to create products and materials from living organisms or biological components. In this context, lignocellulosic fungi cultivated on organic substrates produce biomaterials that are potentially more sustainable in terms of production, use, and disposal making them potentially more sustainable (Vandelook *et al*. 2021). Biomaterials composed of fungal mycelium and lignocellulosic substrates show promising structural and mechanical properties, supporting their application in architecture, packaging, and textiles (Gough *et al*. 2022, 2023). Commercial interest and scientific research into these materials have grown considerably (Vandelook *et al*. 2021).

Ganoderma is a fungus used for biomaterial development due to its rapid growth and non-toxicity to humans and the environment. It is a white-rot fungal genus distributed globally in warm and tropical regions, with ecological, medicinal, economic, and cultural significance in forestry (Moncalvo and Ryvarden 1997; Zhou *et al*. 2015; He *et al*. 2022). It behaves as a parasite on living or decaying trees, degrading lignin and cellulose (Loyd *et al*. 2017). Some species are sources of ligninolytic enzymes for biotechnology, while others contain bioactive compounds used in traditional medicine and pharmacological research (Otjen *et al*. 1987; Dai *et al*. 2009; Hapuarachchi *et al*. 2018a).

Agro-industrial fibrous residues such as coconut husk, pineapple leaves, and sugarcane bagasse represent valuable sources of lignocellulosic biomass for the development of biobased materials. In western Mexico, particularly in the state of Jalisco, the tequila industry generates approximately 400,000 tons of agave bagasse annually as a byproduct of *Agave tequilana* Weber var. azul processing (Gaceta UNAM 2022). According to the Tequila Regulatory Council (CRT), around 600 million liters of tequila were produced in 2023, resulting in considerable volumes of agave bagasse. This residue poses significant environmental management challenges due to its high organic load, but it also offers an opportunity for valorization through alternative uses such as biomaterial production. This approach not only mitigates the environmental impact associated with waste accumulation but also aligns with circular economy principles by promoting resource efficiency and reducing dependence on virgin raw materials.

Several studies have focused on the production of biomaterials using wood-decaying fungi and natural fibers. Gough *et al*. (2024) showed that species such as *Ganoderma lucidum* and *Pleurotus ostreatus* can degrade lignocellulosic substrates and create biocomposites for thermal insulation, coatings, and construction.

Gezer and Kuştaş (2024) inoculated NaOH-treated wheat straw (1%) with *Ganoderma lucidum* to produce insulating panels, which demonstrated good physical and mechanical properties, confirming their viability as insulating materials from lignocellulosic resources. Similarly, Holt *et al*. (2012) developed biodegradable materials from cotton waste using *Ganoderma* sp., with insulating panels showing comparable properties to polystyrene foam. While commercially available *Ganoderma* strains could have been employed, the use of native isolates promotes the valorization of regional fungal biodiversity and locally available biological resources. As this study aimed to evaluate the feasibility of producing mycomaterials from regional agro-industrial residues, a native *Ganoderma* isolate was considered more appropriate. This approach is supported by previous studies highlighting the value of employing diverse fungal species, including native strains, for biomaterial and bioproduct development. For instance, Gutiérrez-Soto *et al.* (2015) demonstrated the selection and characterization of a native *Pycnoporus sanguineus* strain as an effective lignocellulolytic extract producer from submerged cultures of various agro-industrial wastes, underscoring the potential of regional isolates for valorizing local residues. Similarly, Cristini *et al.* (2025) conducted a comparative study of mycelium films from nine fungal species for biocomposite applications, reinforcing the relevance of exploring species diversity—including native strains—to optimize material properties for specific applications.

It was hypothesized that combining agave fibers with a native *Ganoderma* strain would produce a cohesive mycelium-based composite with improved structural integrity and biodegradability compared with the use of either component individually. This hypothesis is based on the complementary roles of lignocellulosic fibers as structural reinforcement and fungal mycelium as a natural biological binder capable of forming an integrated composite without synthetic adhesives.

The aim of this study was to biofabricate sustainable materials from the mycelium of *Ganoderma lucidum* and agave fiber (*Agave tequilana* Weber var. azul), with a primary focus on demonstrating the technical feasibility of transforming these regional agro-industrial residues into mycomaterials. The morphological, chemical, and mechanical properties of these composites were evaluated to explore potential applications and promote circular economy strategies using agricultural residues.

EXPERIMENTAL

Materials

The fungal specimen was collected in the Cerro de Santa María area, Jalisco, Mexico, a protected natural area located in the Tlaquepaque/Santa María Tequepexpan region (approximately 20.569° N, 103.351° W). Strain identification was carried out at the Department of Cellular and Molecular Biology, Universidad de Guadalajara.

The fungus was reseeded and activated using the grain-cultivation technique with sterilized sorghum (*Sorghum* sp.) free of impurities. Two lignocellulosic fibers from agave (*Agave tequilana* Weber var. azul) fibers and maize stover (*Zea mays*) were used. Maize stover was included, as it provides the sugars necessary for fungal development, while agave fiber contributes structural support for consolidating the biomaterial. Preliminary trials were conducted to evaluate different agave:maize stover blending ratios and inoculation rates in order to optimize fiber packing, substrate density, and colonization efficiency. Based on these results, an 80:20 (agave:maize stover) ratio and an 8% (w/w) inoculation rate were selected, as they yielded the most consistent and efficient mycelial colonization. Both fibers were washed, ground, and sieved through a 100-mesh sieve to standardize particle size.

The control material for mechanical testing consisted of commercially available expanded polystyrene (EPS) packaging foam obtained from a local supplier. The bulk density of the EPS was determined gravimetrically and found to be 0.014 g/cm³, corresponding to low-density EPS typically used in packaging applications.

DNA Extraction and Molecular Identification of the Strain

Genomic DNA from mycelial culture was extracted using Wizard Genomic DNA Purification Kit (Promega), Madison, Wisconsin, USA. The ITS1 and ITS2 region of the nuclear rDNA were amplified using the primer pairs G-ITS-F1/ITS5.8S (Cao *et al*. 2012; Vilgalys and Hester,1990) and ITS3/G-ITS-R2 (White *et al*. 1990; Cao *et al*. 2012). Each 53 µL PCR reaction contained 50 µL of PCR mix [35 µL of PCR water, 6 µL of 10X Taq reaction buffer without $MgCl_2$, 3.0 µL of 50 mM MgCl2, 3 µL of 5 mM dNTP, 3 µL of 2 µg/µL Bovine Serum Albumin (BSA)], 0.5 µL of each 10 µM primer, 0.15 µL of Platinum™ Taq DNA Polymerase High Fidelity (5 U/µL), and 2 µL of gDNA template. PCR amplifications were performed in an ESCO Swift MaxPro thermocycler. The DNA was denatured at 95 °C for 3 min, 25 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 45 s, and extension at 72 °C for 2 min. This was followed by 15 cycles of 95 °C for 1 min, 50 °C for 45 s (increasing 5 s each cycle) and 72 °C for 2 min with an extension step of 72 °C for 10 min and final incubation at 15 °C. PCR products were separated by electrophoresis in 1.5% agarose gels with GelRed (Biotium) and visualized in Amersham Imager 600 (GE). PCR products were purified using QIAquick silica columns (PCR purification kit, Qiagen). The purified DNA amplicons were sent to the LaniVeg for Sanger sequencing.

Molecular Identification

The sequences were edited and assembled from the forward and reverse amplicons in Codoncode aligner v. 10.0.2 (CodonCode Corporation, www.codoncode.com). The taxonomic identity of the sequences was determined by submitting the consensus sequences to the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*. 1990) from Genbank, NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/ consulted, January 2026). The criterion for identification was based on the percentage of similarity.

Biofabrication

The biofabrication process consisted of several stages, illustrated in Fig. 1. The following section details the steps involved in the formation of materials and biopackaging. The strain was reseeded from a Petri dish into sterilized glass jars containing clean, hydrated sorghum grains. The jars were incubated for 7 days until complete colonization of the grains was achieved. The hydrated fiber mixture was divided into 700 g batches in autoclavable bags and sterilized at 121 °C for 2 h. After cooling, the mixture was inoculated with *Ganoderma lucidum* at a rate of 8% (w/w) relative to the total fiber weight. Small perforations were made in the bags to enhance aeration and stimulate fungal metabolism. The inoculated mixtures were incubated for 14 days under controlled conditions (65 to 75% relative humidity and 27 to 29 °C) to ensure complete substrate colonization by the mycelium.

Fig. 1. Biofabrication process of *Ganoderma* composites: a) fiber hydration and sterilization, b) inoculation of the fiber with *Ganoderma*, c) mold filling, d) demolding, e) fungal inactivation and biomaterial drying

Subsequently, the colonized mixtures were transferred into metal molds of various geometries and incubated for an additional 7 days. After demolding, the biocomposites underwent a second incubation period to achieve homogeneous colonization. Finally, the composites were dried at 60 °C for 4 days to allow gradual dehydration, minimizing the risk of cracks or deformations.

Properties Evaluation

The morphology of the composites was examined using Scanning Electron Microscopy (SEM) JEOL JSM6610LV, Tokyo, Japan. Prior to imaging, each sample was coated with a thin layer of gold using a sputter coater (Denton Vacuum™ Sputter Coater, Moorestown, New Jersey, USA) to ensure surface conductivity. The samples were then placed in the scanning electron microscope. A representative region of each composite was selected for analysis in order to observe the structure of the mycelium and its interaction with the fibers.

Surface wettability was determined by contact angle measurements employing a Krüss DSA 0031 Drop Shape Analyzer, Hamburg, Germany. This evaluation provided information regarding the material's hydrophilicity, supporting the assessment of its performance under humid conditions and its potential application environments.

Chemical characterization was performed through Fourier Transform Infrared Spectroscopy (FTIR) using a Thermo Scientific Nicolet iS50 spectrometer equipped with an Attenuated Total Reflectance (ATR) module, (Waltham, MA, USA). The spectra were collected to identify the main functional groups present in the mycelium and in the mycelium–fiber composite. Because ATR-FTIR band intensities can be affected by differences in sample–crystal contact, surface roughness, and penetration depth, the spectra were interpreted qualitatively. Therefore, no quantitative comparison based on relative peak heights or peak areas was performed. The discussion was based primarily on the presence, position, and assignment of characteristic absorbance bands.

Mechanical performance (flexural strength, mean force and deflection) was evaluated by three-point bending tests according to ASTM D790-17 using an Mxmoonfree HLB mechanical tester, Hangzhou, China. Rectangular specimens (9×1.8×1.8 cm) were subjected to a maximum load of 500 N until fracture. A total of six replicates were tested for each material.

Bulk density was measured according to ASTM D2395-17 standard to facilitate a comparative analysis with conventional polymeric materials employed in packaging, such as expanded polystyrene (EPS).

Fire resistance was assessed following ASTM D-635-22. Flammability tests were conducted under laboratory ambient conditions (24 ± 2 °C; 55 ± 5% RH) without the use of a combustion chamber. The testing setup, specimen positioning, and the procedure used to determine the burning rate were established according to the standard. Qualitative parameters recorded included flame color, smoke quantity and color, combustion behavior, odor emission, self-extinguishing capability, and dimensional stability after flame exposure.

An environmental performance analysis of the *Ganoderma* composites was conducted utilizing the Life Cycle Design Strategy Wheel (LIDS) proposed by Van Hemel (1998), with EPS serving as reference material. The LIDS framework is based on qualitative criteria that are assigned by the researchers according to the characteristics and available information of the product under study.

Finally, a circular economy model was proposed for the valorization of agave fiber residues, aiming to foster the economic development of tequila-producing communities while mitigating agricultural waste.

RESULTS AND DISCUSSION

Molecular Identification

Two consensus sequences were obtained, one generated with the ITS3 and G-ITS-R2 primers of 580 base pairs (bp) and the other generated with the G-ITS-F1 and ITS5.8S primer pair of 314 bp. The sequences yielded a BLAST identity percentage of 99.65% and 99.24%, respectively, with *Ganoderma lucidum* (Curtis) P.Karst., 1881.

Biofabrication

Figure 2 shows the composites obtained through the mycelial growth of *Ganoderma lucidum* on agave-based substrates. The samples exhibited a well-formed morphology and structural uniformity, consistent with typical fungal-based materials. The versatility of the molding system allowed for the fabrication of functional pieces adapted to various applications. Products obtained included: (a) bottle packaging for the tequila industry, (b) biodegradable pots for horticulture, (c) panels with potential use as thermal or acoustic insulators, (d) eco-friendly bricks, (e) cosmetic product packaging, and (f) multipurpose containers. From a morphological perspective, the materials demonstrated good mold fidelity, well-defined geometries, distinct cavities, and relatively consistent thicknesses, highlighting the versatility of the biofabrication process.

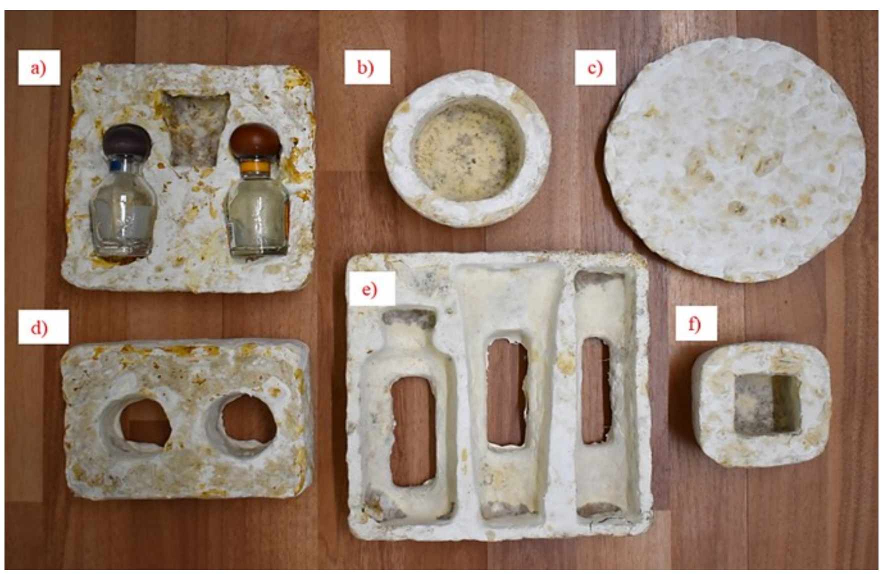

Fig. 2. Different mycelium–fiber composites obtained through biofabrication

Morphological Characterization

The SEM images (Fig. 3) show the fibrous structure of agave and the abundant growth of aerial mycelium. In Fig. 3a, the hyphae formed a dense and interconnected network, indicating effective colonization of the lignocellulosic substrate. Figure 3b reveals fungal hyphae closely associated with the substrate, with numerous filaments adhering to the surface and others penetrating the partially degraded cell walls, as indicated by the yellow arrows. The lignocellulosic fibers exhibited clear signs of deterioration, including loss of structural integrity, collapsed and eroded cell walls, consistent with fungal degradation of lignocellulosic structures. Finally, Fig. 3c displays a dense and entangled network of *G. lucidum* hyphae with a tubular morphology and relatively smooth surface walls. A branched, three-dimensional growth pattern is observed in close contact with agave fibers, indicating effective substrate colonization. The hyphal organization suggests anchoring and expansion behavior that is favorable for biomaterial applications. These morphological characteristics of *Ganoderma* mycelium are consistent with observations reported by Bae *et al*. (2021).

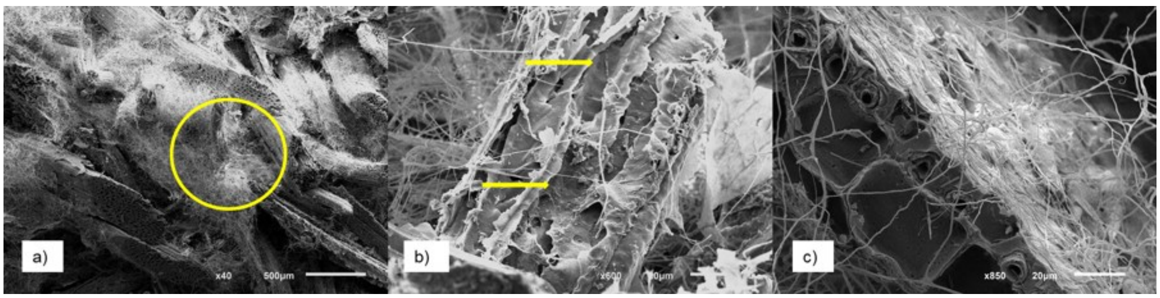

Fig. 3. SEM micrographs of mycelium–lignocellulosic fiber composites: (a) general view of the biomaterial structure, (b) detail of the interaction between mycelium and fibers, and (c) close-up of *G. lucidum* hyphae

Density

The resulting composites exhibited a bulk density of 0.20 g/cm³. This value falls within the typical range reported for polystyrene (0.02 to 0.25 g/cm³) (Yang *et al*. 2015), albeit toward the high end of the stated range. When compared with the results reported by Medina-Gutiérrez *et al*. (2024), who obtained densities ranging from 0.2 to 0.30 g/cm³, this variation may be attributed to differences in the amount of substrate used, which can influence the final product's density. Although the biomaterial's density is higher than that of some expanded polystyrene products, it is still considered lightweight and offers the added benefit of being biodegradable. The slightly higher density may result from a more compact structure in the bio-based packaging, potentially enhancing its mechanical strength without compromising its performance as a packaging material. It is worth noting that low-density materials are generally advantageous for packaging applications, as they facilitate handling, transport, and storage, thereby improving logistical efficiency and reducing costs.

Flammability Tests

Table 1 presents the main parameters evaluated during the flammability tests. Both materials exhibited an orange flame and showed notable differences in their thermal behavior. Images of the flammability tests are shown in Fig. 4. EPS foam exhibited rapid ignition with a combustion rate of 1.22 mm/s until complete consumption, confirming its high flammability and associated fire risk. The flame was accompanied by dense black smoke and a strong, irritating odor. Additionally, the sample experienced dripping of molten material during combustion, indicating low thermal stability. The EPS used in this study was a commercial grade commonly employed for packaging and handicrafts, which is typically not formulated with flame-retardant additives. The behavior observed during testing was consistent with that of conventional EPS, which readily ignites, melts under heat exposure, and produces burning droplets.

Table 1. Flammability Test of the EPS and *Ganoderma* Composites

Material	Smoke		Flame hue	Self-extinguish	Burning speed (mm/s)	Deformation of the specimen	Carbon formation
	Amount	Color		Yes/Not			Yes/Not
EPS	***	Black	Orange	Not	1.2205	Melting	Not
Ganoderma composite	*	White	Orange	Yes	0.0357	None	Yes

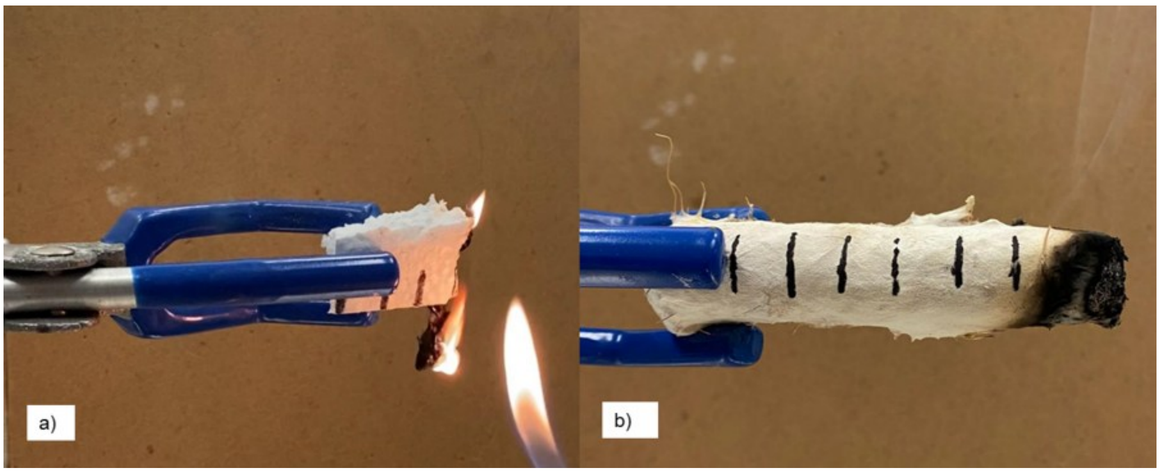

Fig. 4. Video screenshots of flammability tests for a) polystyrene and b) the fabricated biopackaging

In contrast, the composites showed very slow combustion (0.04 mm/s) and self-extinguished at an early stage of the process. They generated less smoke, which was white in color and had a distinctive caramel- and wood-like odor. Unlike EPS, the composites showed no signs of melting or dripping when exposed to flame. Instead, a surface char layer developed that acted as a physical barrier to heat, contributing to its thermal stability. The formation of a char layer (Fig. 4) during combustion is a common feature of materials with high natural fiber content and fungal structures, significantly enhancing fire resistance. This char layer serves as a thermal shield that limits the release of flammable gases and reduces heat transfer, thus slowing flame propagation. In the case of fungal fibers, studies have shown that their chitin-rich composition promotes the formation of carbonaceous residues during thermal degradation (Zhang *et al*. 2022). Similar findings were reported by Jones *et al*. (2018), who demonstrated that mycelium composites exhibit superior fire safety compared to conventional construction materials, with lower heat release rates, reduced smoke production, and delayed flashover; these properties can be further enhanced by silica-rich additives.

Wettability

The sessile drop analysis (Fig. 5) revealed a contact angle of approximately 130°, indicating hydrophobic behavior. This result suggests that the mycelial structure influences the surface properties of the biopackaging. These findings are consistent with those reported by Haneef *et al*. (2017), who observed that mycelium-based materials exhibit water contact angles exceeding 120°, confirming their inherently hydrophobic nature. This behavior has been associated with the presence of hydrophobins in the mycelial cell wall, as described by Sun *et al*. (2020) and Tacer-Caba *et al*. (2020). These proteins are known to play a key role in water repellency, facilitating the development of fungal reproductive structures under humid or aqueous conditions, and promoting initially hydrophobic surfaces with contact angles typically above 90°.

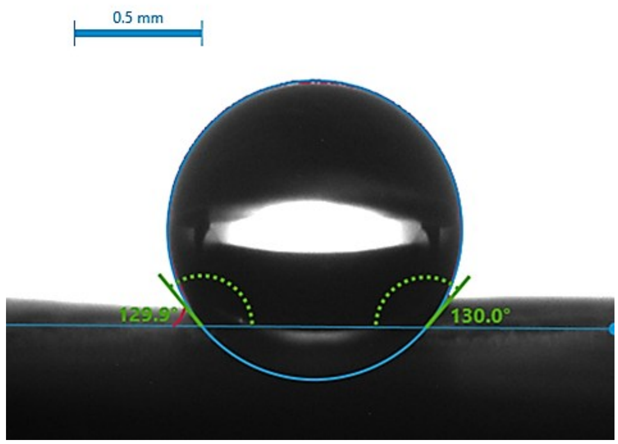

Fig. 5. Representative water contact angle measurement on the mycelium-based composite surface (green). The blue line corresponds to the droplet profile fitted by the image analysis software for contact angle determination

Chemical characterization (FTIR)

ATR-FTIR spectra were used to identify the main functional groups present in *Ganoderma lucidum* mycelium and in the mycelium–fiber composite (Fig. 6). The broad absorbance band observed in the 3600 to 3100 cm^{-1} region was assigned mainly to O–H stretching vibrations from hydroxyl groups present in polysaccharides, including cellulose, hemicellulose, β-glucans, and chitin. Contributions from adsorbed water may also occur in this region. The bands located between 3000 and 2800 cm^{-1} were associated with asymmetric and symmetric C–H stretching vibrations of aliphatic groups present in fungal and plant-derived components. Absorbance bands near 1640 and 1540 cm^{-1} were assigned to amide I and amide II vibrations, respectively, which are commonly associated with proteinaceous materials and chitin-containing fungal structures (Shakir *et al.* 2020). However, the band near 1640 cm^{-1} may also overlap with H–O–H bending vibrations from absorbed water. The fingerprint region between 1200 and 900 cm^{-1} showed characteristic contributions from C–O, C–C, and C–O–C vibrations of polysaccharides, including cellulose, hemicellulose, β-glucans, and chitin. The band near 1040 cm^{-1} was attributed to overlapping carbohydrate contributions from both the fungal biomass and the lignocellulosic substrate, as has also been reported for mycelium-based materials by Haneef *et al.* (2017). Overall, the ATR-FTIR spectra support the coexistence of fungal and lignocellulosic components in the biofabricated composite. The presence of hydroxyl-, amide-, and carbohydrate-related bands is consistent with a polysaccharide-rich system where hydrogen-bonding interactions may contribute to material cohesion. However, due to the qualitative nature of ATR-FTIR under these conditions, the spectra were not used to quantify relative chemical composition or interaction strength.

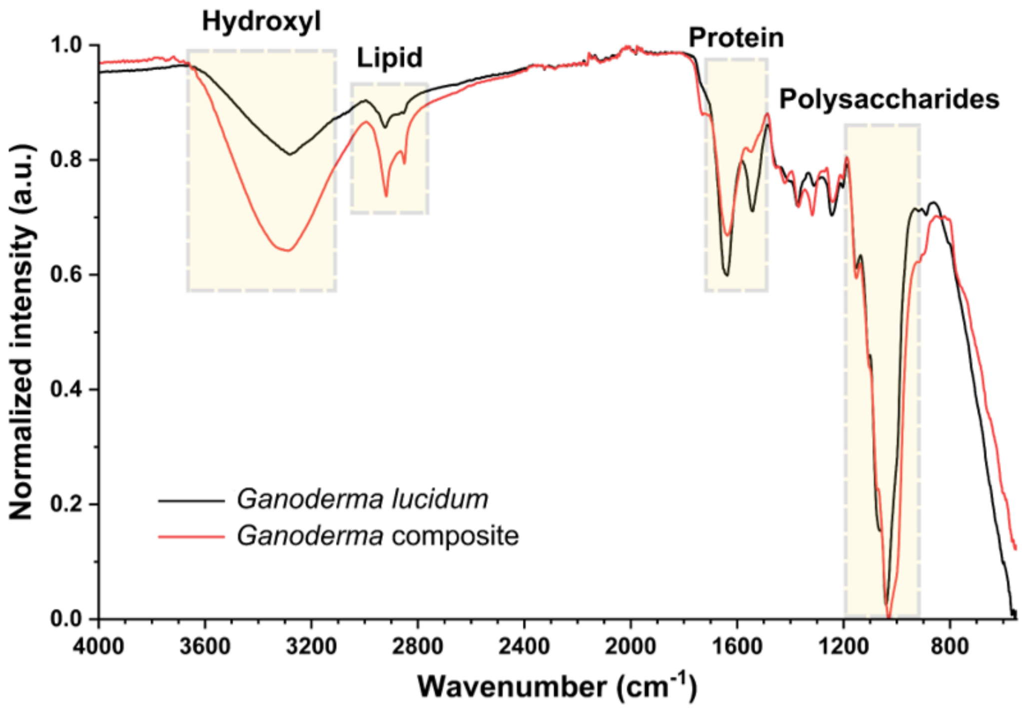

Fig. 6. FTIR spectra of the mycelium of *Ganoderma lucidum* and the *Ganoderma* composite showing the main vibrational bands

Flexural Test

In terms of maximum force (Fig. 7a), the *Ganoderma*-based material reached values close to 8 N. This was slightly below expanded polystyrene (EPS, 8.5 N) yet within the same order of magnitude. The moderate standard deviation suggests a reasonably consistent response, particularly considering the intrinsic variability associated with biologically derived materials. The similarity in peak force indicates that the interconnected mycelial network can effectively transfer compressive stresses through its three-dimensional architecture. For flexural strength (Fig. 7b), the average value for *Ganoderma* composites (0.15 MPa) was lower than that of EPS (0.21 MPa), yet both materials fell within the same order of magnitude and their experimental ranges partially overlap. Lingam *et al*. (2023) reported flexural strengths ranging from 0.0634 MPa in bagasse–rice husk composites to nearly 25 MPa in other species–substrate systems, and Özdemir *et al*. (2022) observed increases from ~10 MPa to 25 MPa with low-density veneer lattice reinforcement. Relative to such findings, the present composites displayed a distinctive response: despite their lower stress capacity, they tolerated considerable bending without rupture. The deformation recorded at maximum load (Fig. 7c) was slightly higher for *Ganoderma* composites than for EPS, 12.33 mm and 11.40 mm respectively, indicating a greater capacity for structural accommodation prior to failure. This higher deflection at similar force levels is consistent with a less rigid, more compliant structure. The combination of moderate load capacity and increased deformability may be advantageous in applications that need controlled flexibility and impact tolerance.

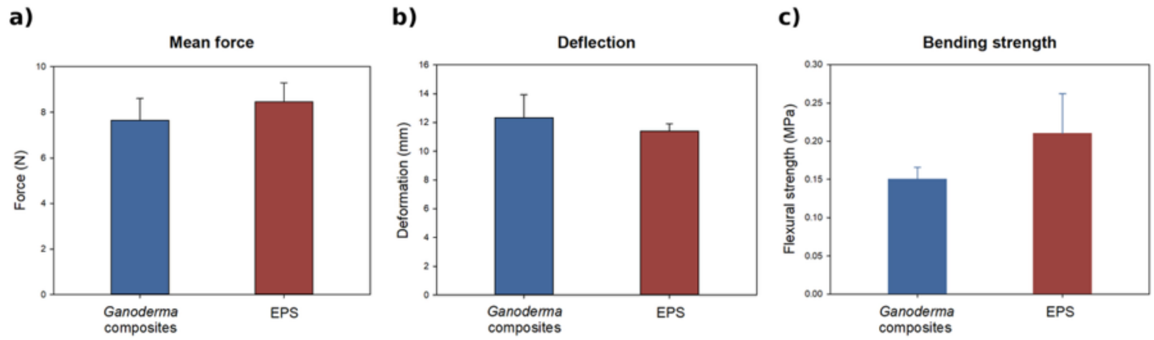

Fig. 7. Mechanical properties of *Ganoderma* composites and EPS

Life Cycle Design Strategy Wheel (LIDS)

This qualitative eco-design tool assesses products throughout their life cycle, from conception to end-of-life. Figure 8 shows the radial diagram derived from the eco-design analysis of the developed biomaterial and EPS, considering eight strategies.

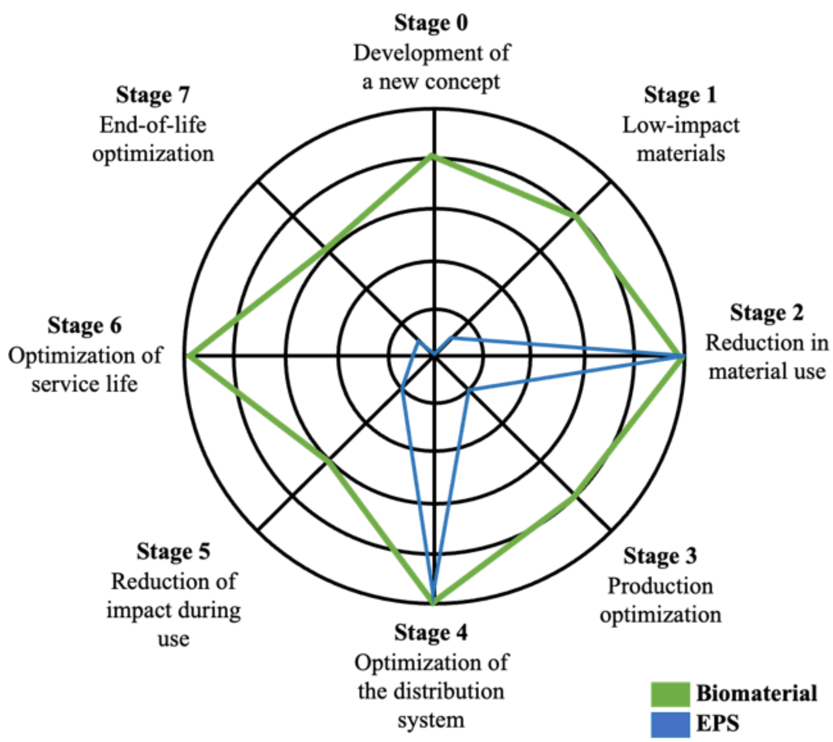

Fig. 8. Eco-design strategy wheel (LIDS) by Van Hemel (1998), comparing the biopackaging (green line) with EPS (blue line)

Each axis was evaluated using an ordinal scale that indicated the level of compliance or improvement of the biomaterial in comparison with conventional EPS. Higher values approaching 100%, and consequently a larger polygonal area, represent a more favorable environmental profile.

Table 2. Comparison of Biomaterial and EPS Performance Based on LIDS Concepts

Stage	Strategy	Description (LIDS concept)	Assigned value – *Ganoderma* composite	Assigned value – EPS
0	Development of a new concept	Promote innovation and novel product functions	80% – Integrates agave bagasse and mycelium; valorizes agro-industrial residues and enables new applications	0% – Conventional product without innovation or waste valorization
1	Low-impact materials	Selection of renewable and non-toxic resources	80% – Produced from natural, local, and non-toxic raw materials	10% – Petroleum-derived, with high environmental impact
2	Material reduction	Minimize the quantity of material required	100% – Lightweight and volume-efficient structure	100% – Also lightweight and volume-efficient
3	Production optimization	Improve efficiency and reduce waste during processing	80% – Potentially lower fossil-resource dependence for mycelium cultivation and a low-waste production process	20% – EPS production requires heat, pressure, and generates emissions
4	Distribution system optimization	Reduce logistics and transportation impacts	100% – Lightweight; potential integration within the tequila production chain to avoid additional transport	100% – Lightweight and easy to transport
5	Impact reduction during use	Minimize energy and resource consumption in the use phase	60% – Requires no energy or inputs during use	20% – No energy required, but provides no additional environmental benefit
6	Life-span extension	Facilitate reuse and prolong product functionality	100% – Durable and reusable in multiple applications	10% – Single-use, with limited reuse potential
7	End-of-life optimization	Improve recycling, reuse, or final disposal options	60% – Compostable under controlled conditions and safe for incineration	10% – Difficult to recycle, slow degradation, and generates toxic compounds upon incineration

The application of the LIDS methodology revealed clear environmental advantages of the biocomposite when compared to its synthetic counterpart. Table 2 outlines the eco-design strategies considered in this evaluation.

The favorable environmental performance indicated by the LIDS analysis should be interpreted with caution, as this framework provides a qualitative assessment based on predefined design strategies rather than a quantitative evaluation of environmental impacts. Since no life cycle assessment or detailed quantification of process energy consumption was performed, the overall energy footprint of the material, including the energy requirements associated with sterilization and thermal inactivation steps, cannot be determined from the present results.

Circular Economy

Tequila production generates large volumes of lignocellulosic residues, whose valorization within a circular economy model could mitigate their environmental impact while enabling the development of high-value-added biodegradable materials. The utilization of agave within a circular bioeconomy framework extends beyond the mere substitution of fossil-based inputs with renewable resources. It requires efficient supply chains and innovative conversion technologies that enable its transformation into bio-based materials, fuels, and products (Tan and Lamers 2021; Pérez-Pimienta *et al*. 2024). Figure 9 illustrates a utilization cycle of agave in tequila-producing communities, highlighting its potential for biopackaging production and soil regeneration through composting.

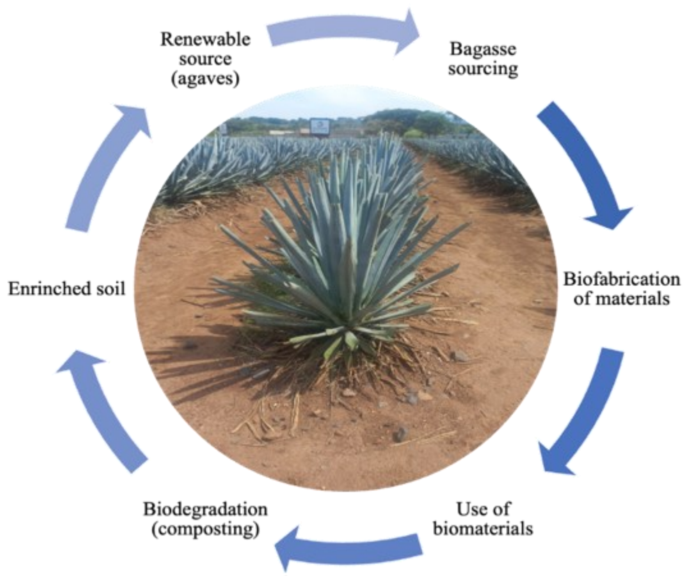

Fig. 9. Proposed circular economy model for tequila-producing communities based on the agave plant

The cycle begins with agave cultivation, which requires several years of growth before harvest. After juice extraction and distillation, the lignocellulosic residues—often regarded as environmental waste—can be transformed into materials applicable in sectors such as packaging or construction, reducing reliance on synthetic polymers.

A circular bioeconomy model seeks to integrate the biological recovery of resources and organic nutrients from waste streams within a circular economy framework (Davis *et al*. 2016; Díaz-Vázquez *et al*. 2022). At the end of their useful life, these biocomposites may be composted, contributing to the return of nutrients to the soil, improving fertility, enhancing water retention capacity, and stimulating microbial biodiversity. This process closes the production loop, allowing enriched soils to support the cultivation of new agave plants, thus ensuring the system's continuity.

CONCLUSIONS

1. The developed biocomposites fall within the range of lightweight materials and offer the additional advantage of biodegradability. Although their density was slightly higher than that of typical expanded polystyrene (EPS), the materials exhibited suitable structural integrity and functional performance, demonstrating their potential as sustainable packaging materials.
2. The morphological analysis revealed good interaction between the fungal hyphae and the agave fibers, resulting in a consolidated and stable biomaterial.
3. The produced materials exhibited hydrophobicity, high resistance to flame propagation, with lower burning rates and reduced structural deformation compared to polystyrene. These characteristics suggest potential applications in contexts requiring good thermal stability and low flammability.
4. Results of attenuated total reflectance-Fourier transform infrared (ATR-FTIR) analysis suggested possible interfacial interactions between the fungal matrix and the agave fibers, as indicated by the broadening of the O–H stretching region and changes in the carbohydrate-rich fingerprint region. These spectral features are consistent with hydrogen-bonding interactions and polysaccharide overlap, which likely contributed to the cohesion of the composite.
5. The *Ganoderma*-based composites exhibited a balanced mechanical performance characterized by consistent load transfer through the interconnected mycelial network and a notable capacity for deformation prior to failure. Although their maximum force and flexural strength were within the same order of magnitude as EPS, the composites distinguished themselves by tolerating greater deflection under comparable loading conditions. This behavior reflects a compliant three-dimensional architecture capable of redistributing stresses without abrupt rupture. The combination of structural integrity, reproducible response, and enhanced strain accommodation highlights the potential of these bio-derived composites for applications where energy absorption, impact mitigation, and controlled flexibility are advantageous.
6. The qualitative evaluation using the LIDS tool showed a more favorable environmental performance of the agave–mycelium biocomposite compared with expanded polystyrene, particularly regarding the renewable origin of the raw materials, production efficiency, and end-of-life options. The eco-design approach proved effective in identifying opportunities for environmental improvement throughout the product life cycle, from raw material selection to post-consumer valorization.
7. Finally, the efficient utilization of agro-industrial by-products not only supports local economic development but also strengthens the sustainability of the tequila sector by closing production loops and fostering a regenerative approach within the industry.

FURTHER STUDIES

The results obtained in this study demonstrate the potential of agave fiber and *Ganoderma lucidum* mycelium for the development of bio-based composite materials. Building on these findings, several directions merit further investigation. First, the mechanical characterization of the developed mycomaterials should be expanded through additional testing to provide a more comprehensive evaluation of their performance and potential applications. Second, translating the laboratory-scale parameters established here (substrate ratio, inoculation rate, and colonization conditions) into a modular, parallelized production scheme would be a key step toward industrial implementation, supported by techno-economic and process optimization studies to evaluate feasibility at larger scales. Finally, from a circularity perspective, the potential for reuse of the bio-based packing material prior to final disposal should be explored, as a multi-use cycle followed by biodegradation could offer a more circular alternative than single-use disposal. End-of-life scenarios should also be compared under different disposal conditions (*e.g.*, aerobic composting versus anaerobic soil burial) to assess their relative environmental impact, including methane generation potential.

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