RESEARCH ARTICLE

Sustainable Mycelium-Based Leather: Conversion of Bread Waste into Fungal Biomaterial Using Vegetable Tannins

S.T.Gopukumar[1*], Nithya Rathinam[2], N.Prabha[3], V.Ravikumar[3], A.Ganesh Kumar[4], P.Sankarganesh[5], G.Manivannan[6], Jeniffa Rajavedhanayagm[7]

[1]Nanobioinformatics Unit, Department of General Surgery, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai – 602 105, Tamil Nadu, India

[2] Department of Ophthalmology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai – 602 105, Tamil Nadu

[3]Department of Microbiology, SRM Arts and Science College, Kattankulathur, Chengalpattu District – 603 203, India

[4]Department of Microbiology Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai – 602 105, Tamil Nadu, India

[5]Oculo-Nutri Interdisciplinary Lab, Department of Ophthalmology, Saveetha Medical College andHospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

[6]Research Unit, Department of Psychiatry, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai – 602 105, Tamil Nadu, India

[7]Department of Mechanical and Aerospace Engineering, The Ohio State University, N350 Scott Laboratory, 201 W 19th Avenue, Columbus, OH 43210

ABSTRACT: The growing market need for sustainable cruelty-free materials enables research into fungal-based leather production as an animal leather substitution. The researchers studied fungal cultivation of Aspergillus niger on bread waste using solid-state fermentation to extract vegetable tannins from sawdust which became the base material for fungal leather production. Mycelial mats reaching high density were collected for tannin processing that improved their mechanical stability. The presence of tannins was confirmed through both qualitative and quantitative analysis while FTIR and SEM helped identify functional group behavior and appearance characteristics. The analysis using Thermogravimetric Analysis (TGA) showed better thermal stability after the treatment step. The experiments also measured tensile strength and density alongside elongation at break. Researchers demonstrate through this study that food waste can be effectively used for developing environmentally sustainable fungal leather while also helping waste valorization and preserving the environment.

Keywords: Aspergillus niger, Bread Waste, Biomaterials, Fungal Leather, Sustainability, Vegetable Tannins.

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1. INTRODUCTION

Sustainability ensures natural and physical resource availability by preventing depletion. Eco-friendly, vegan, and cruelty-free products are crucial in the fashion and luxury industries to reduce environmental impact and protect ecosystems. These organizations must make strategic and operational changes to decrease waste, recycle materials, and develop sustainably at all levels to embrace sustainability [1]. Climate change, population expansion, and resource scarcity threaten the luxury industry, which relies on natural resources, and could affect material supply and prices. Fashion firms must prioritize natural resource conservation, regenerative techniques, and sustainable raw materials to address this [2]. Leather manufacturers, which pollute owing to traditional tanning, must adopt cleaner technology. Waste management and sustainable solutions like recycling and chemical reduction can reduce environmental damage and help the industry become more sustainable [3].

Environmental issues plague the leather

**Corresponding Author:gopukumars.smc@saveetha.com*

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industry, projected at $0.53 trillion in 2024 with a 7.58% CAGR through 2028, notably in leading producers like India, Italy, Korea, and China. Leather manufacture, which requires considerable chemical processing, produces 30 liters per kilogramof cleaned effluent and about 170,000 tonnes of chromium waste annually [4]. Tanneries in India produce 30 billion gallons of wastewater comprising organic contaminants and chromium. Recycling and employing biological treatments like enzymes help reduce hazardous emissions, but more innovation is needed to address these environmental concerns [5].

Nature has full of Aspergilli species, including *Aspergillus niger*, that thrive on litter, soil organic waste, decomposing plant material, and compost. A. niger may survive at 6–47°C, but it prefers 35–37°C. Its water activity growth limit is 0.88 and it can survive in pH 1.4 to 9.8. These qualities increase its occurrence, especially in warm, humid climates. Most fungi establish a mycelial network by expanding hyphae. This network of branching hyphal cells makes fungi a promising textile option for clothes and leather. Sustainable leather-like replacements can be made from filamentous fungus. Upcycling agricultural waste into affordable and versatile leather alternatives improves resource efficiency and reduces waste [6].

The global push for sustainability has intensified the search for alternative materials and the valorization of waste resources [7]. Food waste, a major environmental challenge, offers untapped potential as a substrate for microbial cultivation [8]. *Aspergillus niger*, a filamentous fungus with diverse industrial applications, presents a promising avenue for converting food waste into high-value biomaterials [9]. This study explores the isolation, identification, and cultivation of *Aspergillus niger* from food waste, focusing on its ability to grow on bread waste as a substrate under solid-state fermentation conditions. The research aims to address waste management issues while demonstrating the feasibility of fungal biomass production as a sustainable resource.

2. MATERIALS AND METHODS

2.1. Isolation and Cultivation of Aspergillus niger

Laboratory personnel obtained Aspergillus niger from spoiled strawberry fruit and grew the sample on potato dextrose agar (PDA) before identifying it using lactophenol cotton blue staining and 18S rRNA sequencing [5]. Researchers spread fungal spores across sterilized bread waste adjusted with moisture before keeping the materials at room temperature for 15–18 days under solid-state fermentation conditions.

2.2. DNA Extraction and Molecular Identification

2.2.1. DNA Extraction

By employing the CTAB technique, genomic DNA of superior purity was isolated from fungus biomass. After suspending a loop of fungal material in 400 µL of CTAB buffer, 20 mg/mL of Proteinase K was added to degrade the protein components. To guarantee full lysis, the mixture was kept in an incubator at 55°C for two hours. After that, phase separation was achieved by treating the supernatant with a chloroform: isoamyl alcohol (24:1) mixture. After DNA was extracted with isopropanol, it was rinsed with 70% ethanol, allowed to air dry, and then resuspended in elution buffer for further testing.The genetic extraction of genomic DNA depended on CTAB methodology which led to successful ITS amplification using ITS1 and ITS4 primers. A sequencing process identified the species through BLAST analysis while phylogenetic trees helped confirm the results [6].

2.2.1. Quantification and Amplification

The quality and quantity of DNA were assessed using a Nanodrop spectrophotometer. The ITS region of the fungal DNA was amplified using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR conditions included an initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation (94°C, 30 seconds), annealing (56°C, 30 seconds), extension (72°C, 45 seconds), and a final extension at 72°C for 7 minutes. Amplified products were analyzed using agarose gel electrophoresis.

2.3. Preparation of Bread Waste Substrate

Bread waste was dried before becoming powdered then brought to 60% moisture and later received 121°C heat sterilization for 15 minutes. SSF conditions came into use when A.

niger spores received inoculation onto the prepared substrate.

2.3.1. Fungal Cultivation

Sterile bread substrate was inoculated with spore suspensions and incubated under solid-state fermentation conditions at room temperature for 15–18 days. Thick mycelial mats were formed, harvested, rinsed, and dried for further analysis. Optimal yield was assessed while fungal development was tracked throughout culture. The development of thick, fibrous mats was one of the morphological traits that were noted. Molecular tests of the produced biomass verified the strain's identity and demonstrated its capacity to grow on substrates made from food waste. This methodical strategy helped the study prove that using food scraps for long-term fungal cultivation was feasible by making sure the experimental techniques could be repeated and relied on.

2.4. Tannin Extraction and Characterization

The extraction of vegetable tannins from sawdust through the Soxhlet method under 70°C temperature for 4 hours occurred with distilled water acting as the solvent. The presence of tannin in the extract was verified using qualitative ferric chloride and gelatin tests as well as quantitative UV-Vis spectrophotometry and Folin-Ciocalteu method analysis [7].

2.4.1 Qualitative - Ferric Chloride Test

Sawdust extract can have its tannin content determined using the phytochemical test, a qualitative technique. To do this test, we add ferric chloride, which changes colour as it combines with phenolic chemicals. A change in colour from green to a dark blue or blackish hue indicates the presence of phenolic compounds, including polyphenolic tannins.

2.4.2 Qualitative - Gelatin Assay

One millilitre of the extract was put to a test tube along with one millilitre of 1% gelatin solution and sodium chloride. Tannins are present in the extract if they cause a precipitate to develop [7].

2.4.3. Quantitative Analysis

One millilitre of the extract was placed in a test tube, followed by the addition of one millilitre of a 1% gelatin solution and sodium chloride. The production of a precipitate signifies the presence of tannin in the extract upon achieving a constant weight; the sawdust extract is transferred to a pre-weighed and dried beaker, maintained at 105±2°C in an oven. The extract is measured post-cooling. Subsequently, a 1:1 (v/v) solution of formaldehyde and HCl is incorporated into the extract and permitted to dissolve completely for four hours. Subsequently, hot distilled water is employed to rinse the solution following its filtration through filter paper. Following a two-hour air-drying period, the resulting precipitate is weighed.

2.4.4. Quantitative Determination of Tannin

Once the sawdust extract has reached a steady weight, it gets transferred to a beaker that has been previously weighed and dried in an oven set at 105±2°C. The extract is weighed after cooling. After that, a 1:1 (v/v) solution of formaldehyde and HCl is added to the extract, and it is allowed to dissolve fully for four hours. After that, hot distilled water is used to rinse the solution after it has been run through filter paper. After being allowed to air dry for two hours, the resultant precipitate is weighed.

2.5. Fungal Leather Production

The mycelial mats received tannin extract treatment at 1:0.45 ratios with dry mass before receiving glycerol for improved flexibility during an 8-day incubation period. The sheets required drying while resting under standard weights to achieve uniform surface texture.

2.6. Mechanical and Thermal Property Analysis

Tensile strength measurements occurred via standard shape testing using 1 mm/min stress at a tensile tester [8].Drying the samples at 105°C followed by weight measurement and thickness evaluation served to calculate density [9].

The FTIR analysis of tannin-fungal biomass functional group bonds occurred through Fourier Transform Infrared Spectroscopy (FTIR).Morphological structures of fungal leather received investigation through the use of Scanning Electron Microscopy (SEM).The weight loss measurements during TGA took place under

nitrogen gas flow from room temperature to 1000°C at a 10°C/min heating rate [10].

3. RESULTS

3.1. Isolation and Identification

Aspergillus niger was successfully isolated from spoiled strawberry fruit. Colonies displayed initial white cottony growth, transitioning to black spore heads with pale-yellow undersides. Microscopic examination revealed septate hyphae and conidia characteristic of Aspergillus species. Radial fissures in the agar medium were visible on the colonies' pale-yellow backside. These physical traits led to the identification of the fungal isolate as *Aspergillus sps* (Figure.1).

Figure 1.*Aspergillusniger*

3.2. Molecular Confirmation

DNA extraction yielded high-quality genomic material with an A260/A280 ratio of 1.8, indicating purity. Amplification of the ITS region produced clear bands on agarose gel. The amplicon that was produced displayed a similarity index that was in line with accepted reference standards (Figure 2). BLAST analysis of the sequenced ITS region confirmed the isolate as *Aspergillus niger*. Phylogenetic analysis demonstrated close evolutionary relationships with reference strains. Phylogenetic analysis using combined ITS sequence data from various Aspergillus taxa revealed that the final alignment showed the highest similarity with 78 hits. BLAST analysis in the NCBI database identified the isolate as *Aspergillus niger*.

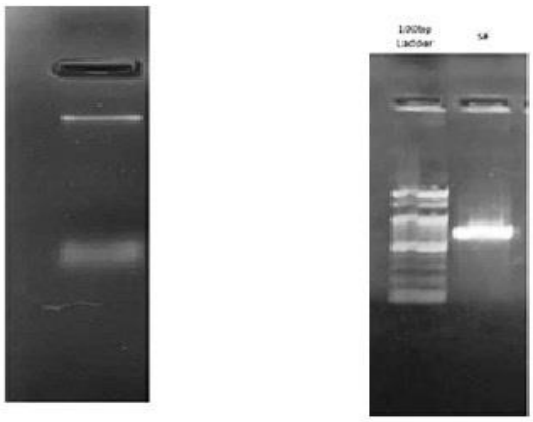

Figure 2: Fungal Genomic DNA & PCR products of ITS gene region

3.3. Fungal Cultivation

Solid-state fermentation on bread waste produced dense, interconnected mycelial mats within 15–18 days. The substrate's high carbohydrate content supported robust fungal growth, highlighting its suitability as a sustainable nutrient source. After 15–18 days, a cottony, dense, and robust fibrous growth was observed on the medium (Figure. 3&4). *Aspergillus niger* is a rapidly growing fungus, and its cultivation on bread waste was previously documented [10].

Figure 3: Stale bread as substrate

Figure 4: Bread waste as substrate in SSF

3.4. Tannin Extraction

Soxhlet extraction yielded 250 mL of tannin solution (Figure 5), with qualitative tests confirming the presence of tannins (Figure 6 & 7). Quantitative analysis showed characteristic absorbance peaks (350–500 nm), and the Folin-Ciocalteu method measured total tannin content as 25 mg/g.

Figure 5: Final extract

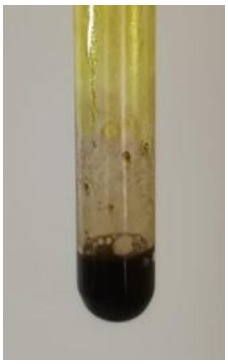

Figure 6: Ferric chloride test (positive for tannin)

Figure.7:Gelatin test (positive for tannin)

3.5. Spectrophotometric analysis

The peaks observed in the prepared extract were within the range of 390–490 nm, characteristic of tannins due to their typical occurrence between 350–500 nm (Figure. 8).

3.6. Mechanical Properties

Table 1 show that the prepared sheets' characteristics are much lower than native leather's, with values of 141 kg/cm^2, 32 kg/cm^2, and 9%. Despite this, they may continue to serve as a biomaterial with applications in biofabrication and tissue engineering. Previous research by C.P. Barnes in 2007 found that native leather goods had tensile strength of 150 kg/cm2, ripping strength of 32 kg/cm2, and elongation at break of 40%, as shown in Table 1. The identified functional group interactions between tannins and fungal biomass, including

carbonyl bonds are the indicative of enhanced cross-linking.

According to the Young's modulus data from Mat Web (Table 1& 2), the sheets have a density of 150 g/cm^3, which is in accordance with the norms for lightweight leather and hard polymer foams like ABS.

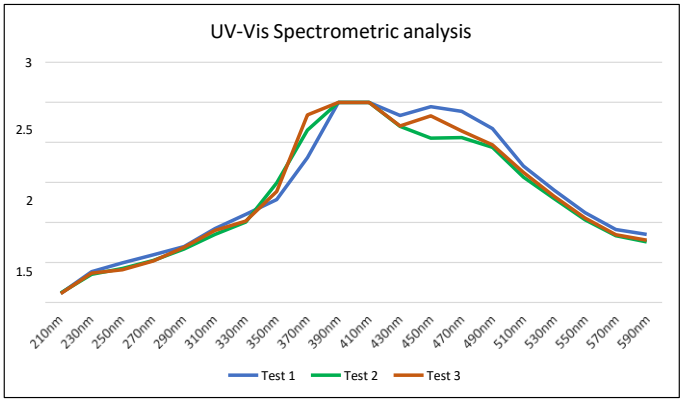

Figure 8: Graphical representation of UV-Vis Spectrophotometric peak of tannin

Table 1: Result of comparison of mechanical properties between conventional leather requirements and prepared sheet

Leather properties	Required value	Prepared sheet
Tensile strength[kg/cm^2]	150 (minimum)	141
Tearing strength [kg/cm^2]	30 (minimum)	32
Percentage of elongationatbreak(%)	40 (minimum)	09

Table 2: Results of the measurement of the prepared sheet

Weight (g)	Length(cm)	Width(cm)	Thickness(cm)
30	1	1	0.2

3.6.1 Fourier - Transform Infrared Spectroscopy

There were more than five peaks seen, although the ones at 1857.45 cm^{-1} and 1992.47 cm^{-1} did not seem to be very powerful. The triple bond area peak (2112.05 cm^{-1}) was weak, and no unique peak for aldehyde was seen. A carbonyl (C=O) bond was determined by the detection of a strong and sharp peak at approximately 1610.56 cm^{-1} (Table 3 & Figure 9).

Table 3: FT-IR functional group database

S.no	Peak	Intensity	Group	Class	Peak details
1	532.35	15.51	C-Brstretching C-Istretching	Halo compound	Strong

2	1022.27	17.11	C-Fstretching	Fluoro compound	Strong
			C-Nstretching	Amine	Medium
3	1217.08	28.97	C-Fstretching	Fluorocompound	Strong
			C-O stretching	Alkylarylether	Strong
			C-N stretching	Amine	Medium
			C-O stretching	Vinylether	Strong
4	1527.62	30.92	N-Ostretching	Nitro compound	Strong
5	1610.56	31.81	C=O stretching	δ-lactam	Strong
			C=C stretching	Conjugated alkene	Medium
			N-H bending	Amine	Medium
			C=C stretching	Cyclic alkene	Medium
			C=C stretching	α,β-unsaturated ketone	Strong
6	1857.45	58.05	C-H bending	aromaticcompound	Weak
7	1992.47	67.02	N=C=Sstretching	Isothiocyanate	Strong
			C=C=C stretching	Allene	Medium
			C-H bending	Aromatic compound	Weak
8	2112.05	54.32	C≡Cstretching	Alkyne	Weak
			N=C=Sstretching	Isothiocyanate	Strong
09	2397.52	41.34	O=C=Ostretching	Carbon dioxide	Strong

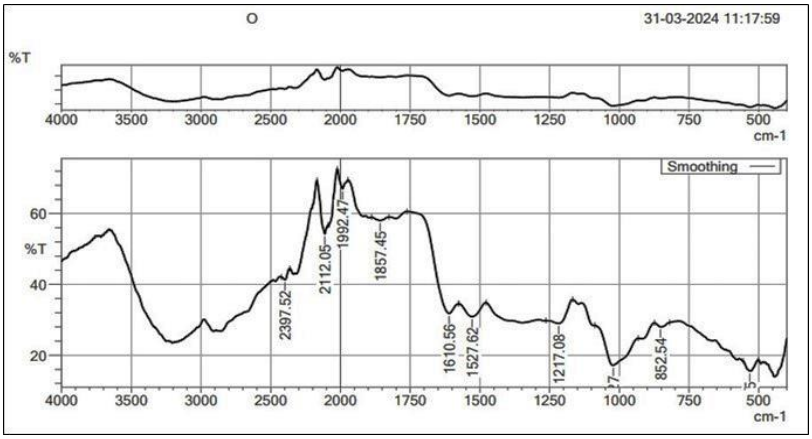

Figure 9: FTIR analysis represented as a graph with peaks

3.621. Morphological and Chemical Analysis

3.6.2.1. SEM:

SEM images revealed a surface with densely packed hyphae on the prepared sheets, along with nano/microfibers and a highly branched hyphal network (Figure. 10). Revealed a dense hyphal network and nano/microfibers enhancing structural integrity.

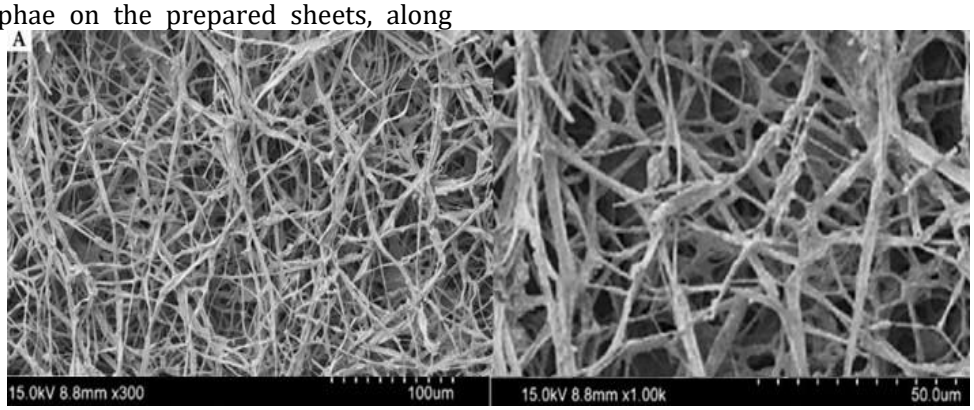

Figure 10: FE-SEM images of the surface morphology of prepared sheets

3.6.2.2. Thermal Stability

The temperature stability between 30°C and 1000°C was checked with TGA analysis. The mass loss-temperature profiles from the TGA showed three different stages. However, there were no noticeable changes in the thermal degradation properties of the mycelial mat(Figure 11). During the first phase of degradation, between 30 and 150°C, 9.70% of the mass was lost. This was due to the evaporation of both free and chemically linked water (H_2O). More secondary mass was lost (45.89%) between 200 and 375°C. This was likely because organic materials like chitin, carbohydrates, and amino acids broke down. At 325°C, half of the weight was lost. During the last step of decomposition (450–600°C), the residual carbon broke down even more, causing a 19.14% mass loss, the production of methane (CH_4), and the formation of residual carbon.

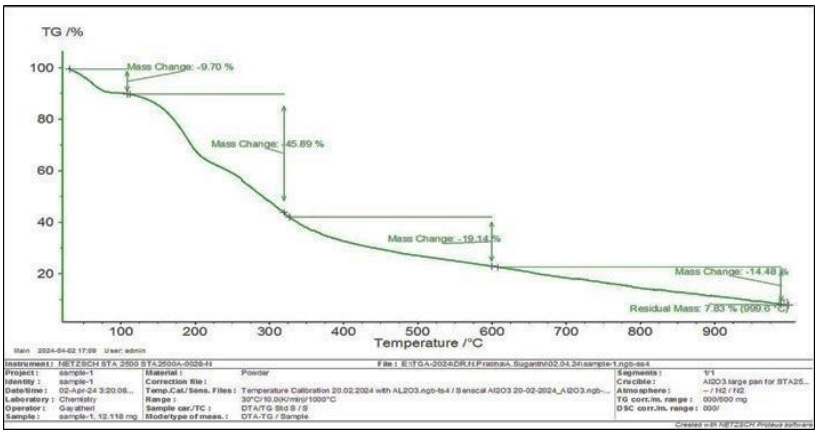

Figure 11: Thermogravimetric (TG) curves for the sheets obtained at a heating rate of 10 °C/min

4. DISCUSSION

This study demonstrates the feasibility of using bread waste as a substrate for the cultivation of *Aspergillus niger*, contributing to sustainable waste management practices. The isolation and identification methods ensured strain specificity, while the cultivation process highlighted the adaptability of *Aspergillus niger* to nutrient-rich food waste substrates. The

results align with previous research on fungal growth optimization, supporting the potential of microbial systems in addressing global sustainability challenges. By converting food waste into fungal biomass, this study provides a practical solution for reducing waste and creating value-added products. The fungal growth on bread waste highlights the substrate's suitability due to its rich carbohydrate composition, aligning with the principles of solid-state fermentation for efficient biomass production [7]. Furthermore, the molecular identification techniques employed here ensured the precise identification of the fungal strain, reinforcing the reliability of this approach for microbial studies [9].

The results are consistent with global efforts to valorize food waste and create value-added products, as seen in similar studies that emphasize the conversion of agro-industrial waste into economically viable and environmentally friendly resources [8]. This approach aligns with the circular economy framework, emphasizing waste reduction, resource efficiency, and sustainability. The integration of microbial systems in addressing food waste challenges not only mitigates environmental issues but also opens avenues for the development of innovative biomaterials [10-13].

This study successfully demonstrates the feasibility of producing fungal leather using mycelium and vegetable tannins. The integration of bread waste as a substrate addresses food waste management, while the use of tannins provides an eco-friendly alternative to traditional chemical tanning. The mechanical properties, though slightly lower than conventional leather, meet the requirements for light leather applications. Based on Blainski, tannin content was calculated [14]. Using the Folin-Ciocalteau colorimetric method, tannin concentration was determined at 25 mg/g, yielding 750 mg per 30 g. The findings of 141 kg/cm^2, 32 kg/cm^2, and 9% suggest that the produced sheets have much poorer characteristics than natural leather. They could be used in tissue engineering and biofabrication as biomaterials. It was to be found 150 kg/cm2, 32 kg/cm2, and 40% elongation at break in native leather goods [15]. In most practical situations where leather with precise physical qualities linked to density is needed and it found that apparent density is more essential than genuine density [16]. The sheets have a density of 150 g/cm^3, meeting criteria for light leather and hard polymer foams like ABS based on Young's modulus data from Mat Web.

CONCLUSION

The study proves bread waste functions as an environmentally friendly substrate for *Aspergillus niger* cultivation. The fungus shows great potential as it successfully passes through identification steps and achieves optimized growth to convert food waste into fungal biomass. The findings advance both sustainable industrial biotechnology and eco-friendly biomaterials through principles of circular economy. The research implements microbial waste transformation strategies which serve as both a waste management solution and an environmental resource efficiency tool.

The researchers should prioritize industrial-scale development of this process for future investigations. Food waste filamentous fungus may replace animal-derived collagen in leather-like products. Successful vegetable tanning was followed by post-treatments to improve characteristics. These fungi are becoming vital for sustainability, pollution-free environments, and renewable fossil-based resource replacements [17]. Glycerol gave softness, while the binder gave it a glossy finish and increased aesthetics. Fungal submerged growth on bread waste scaled up successfully, proving its viability. Fungal leather manufacture also used biotechnology, leather, and paper procedures [18]. The fungal leather substitute industry is expected to grow due to global environmental sustainability, the use of cheap agricultural byproducts as fungal feedstock, eco-conscious fashion trends, and supportive laws and regulations. The development and characterization of fungal leather using vegetable tannins offer a sustainable alternative to conventional leather. The process integrates waste utilization, biotechnology, and traditional tanning methods to produce a material with promising mechanical, morphological, and thermal properties. This innovation supports eco-conscious industries and promotes environmental sustainability [19]. Future research will explore scalability, additional treatments for improved properties, and broader applications in various industries.

Conflict of Interest
The authors declare no conflict of interest.

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The authors have no relevant financial or non-financial interests to disclose.

Data Availability
All the data is available with the authors and shall be provided upon request.

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