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„Development and characterisation of textile materials derived from fungal biomass“

verfasst von / submitted by

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Preface

First, I would like to express my gratitude to my supervisor Prof. Alexander Bismarck for giving me the opportunity to work on such an exciting and creative research project. I am very thankful for the knowledge and experience I gained during this period. Also, I want to thank Momo not only for providing scientific guidance but also for the fun moments in the lab, her words of encouragement and constant support. Furthermore, I want to thank the entire PaCE group for being so welcoming and supportive throughout my stay.

In addition, I would like to thank my family and friends for all their support and love during my whole studies. Thank you for always being there for me!

Inhaltsverzeichnis

Abbreviations

A	Area [m^2]
ANOVA	Analysis of variance
CG	Chitin-glucan complex
d	Thickness [m]
DM	Dry mass [%]
EtOH	Ethanol
gsm	Grams per square meter [g/m^2]
m	Mass [g]
m_D	Mass after drying [g]
m_l	Mass of retained liquid [g]
m_t	Mass of dry textile [g]
m_w	Mass before drying [g]
NaOH	Sodium Hydroxide
Φ	Porosity [%]
PU	Polyurethane
ρ_e	Envelope density [kg/m^3]
ρ_s	Skeletal density [kg/m^3]
SSF	Solid State Fermentation

1. Introduction

As the world's second-largest polluting industry, the textile industry immensely impacts the environment by using unsustainable fabrics and the involvement of many polluting production processes.[1] Apart from the quantity of harmful chemical waste generated in many steps of the manufacturing process, fast-changing fashion trends lead to short apparel life cycles and an increasing amount of textile waste.[1,2] With the rising environmental consciousness of society, sustainable fashion has become an essential topic in the textile industry.[2] Thus, the need to reduce the environmental impact of the textile industry resulted in an increasing demand for environmentally responsible fabrics derived from renewable resources.[2]

Fungi-derived materials have recently raised great interest in the production of fabrics, which is due to their environmental compatibility and unique mechanical properties.[3,4] Fungi-based fabrics mainly comprise structural biopolymers, such as chitin, chitosan and β-glucan.[3,4] Therefore, these materials are fully biodegradable and a promising sustainable alternative to conventional textiles.[3,4] The focus of this work was set on the development of leather-like materials and cosmetic sheet masks derived from fungal biomass. This is done by extracting chitin, chitosan and β-glucan from fungal fruit bodies, followed by film preparation methods.

1.1. Fungi-based leather-like materials

1.1.1. Conventional leather materials and their environmental impact

Leather is a naturally derived product obtained through the chemical and physical treatment of animal skin.[5] Owing to its excellent mechanical properties, unique haptic, and aesthetic functions, leather is used in various application areas, such as in the clothing, footwear and furniture industries.[3] In 2022, the global market size of leather goods was estimated at USD 242.85 billion and the industry is expected to continue to grow.[6] Despite their biodegradability, leather is not only associated with ethical issues but also its high environmental impact, mainly contributed by livestock rearing.[3] With emissions estimated at 7.1 gigatonnes CO_2 equivalents per year, the livestock sector represents 14.5 % of all anthropogenic greenhouse gas emissions.[7] Especially cattle farming is responsible for the high contribution to global greenhouse gas emissions due to the considerable methane emissions generated during farming and from animal manure.[8] Additionally, the livestock sector is associated with deforestation, leading to high land use changes and the loss of biodiversity and carbon storage.[3,9] Another problematic aspect of leather production is the amount of water and hazardous chemicals required during leather treatment, the safe disposal of which remains unresolved.[5] These issues resulted in the demand for leather substitutions, such as synthetic leather materials made from polyurethane (PU) and polyvinyl chloride.[3] Synthetic leather materials eliminate the ethical conflicts and the environmental impact of the livestock sector, making it a more sustainable alternative than natural leather.[3] However, polyurethane and polyvinyl chloride are derived from fossil resources and are not biodegradable, severely limiting end-of-life possibilities.[10,11] This increases interest in biodegradable artificial leather, leading to extensive research of materials derived from biological and renewable sources.[3] Recently, leather material derived from fungi is emerging as a promising substitution candidate in this field.[3,4]

1.1.2. State of the art: fungi-based leather substitutes

Due to their environmental responsibility, fungi-based materials rapidly gained high attraction as a new competitor for leather substitutions.[4] Using fungi as a manufacturing platform can offer several advantages, including its environmental friendliness and cost-efficient cultivation method.[4] Fungi can be grown under ambient conditions with low energy requirements while upcycling agricultural waste that can be used as growth substrates.[4] Thus, several fashion and designer platforms recently focused on using fungi-based materials as a leather alternative.[3] Unlike bovine and synthetic leather, fungi-based leather substitutes do not require hazardous chemicals during production and processing and are also fully biodegradable at the end of service life if not hybridised with other materials.[3] The most popular approach for producing fungi-derived leather-like materials is solid-state fermentation (SSF), where mats of fungal mycelium are grown on the surface of a solid substrate. [4] After harvesting, the mycelium mat has to undergo chemical and physical treatments to achieve the desired leather-like haptic and surface properties.[4]

Mycelium-based leather imitates are already available on the market, such as Reishi™, a leather-like material based on Reishi mycelium from MycoWorks.[12] These mycelium-based leather alternatives are quoted to show tensile strength of 5.6-12.5 MPa with strain to failure at 16-80 %.[12] Bovine grain leather typically has tensile strength between 7-22 MPa and max.

tensile strains between 35-56 %.[13] Tensile strength of artificial leather, such as PU leather, reaches up to 13 MPa with strain to failures between 15-60 %.[14,3] Drawbacks of the SSF are the specific and strict cultivation conditions, such as elevated temperatures, controlled humidity and high carbon dioxide concentrations, which are essential to prevent the formation of fruiting bodies.[3,15] Furthermore, the dependency of SSF on the nutrient profile and density of the inoculum can result in mycelium mats with heterogeneous structures and uneven properties such as thickness, density and morphology.[16,4] Furthermore, scalability, which is crucial on an industrial scale, is also highly restricted. This is because such specific cultivation conditions are challenging for larger systems due to the buildup of temperature, pH, gas, and nutrient gradients.[16,4]

To overcome the disadvantages of mycelium-based leather-like materials, this project focuses on developing leather substitutes by utilising fungal fruiting bodies. This is done by extraction of chitin-glucan from fungi and chitin-glucan film preparation. The mechanical properties should be improved by reinforcement with fibres. Ideally, the used fibres should derive from renewable sources and not affect biodegradability. In this project, lyocell and mulberry bark fibres were tested for reinforcement. Lyocell fibres are produced by the direct dissolution of cellulose. [17] It is known to have excellent dry and wet tensile strength, be comfortable to wear, and be easy to dye.[17] Mulberry bark fibres derived from mulberry trees are similar to cotton constituting cellulose, hemicellulose, lignin and wax.[18,19] Unlike cotton, it also contains gum substances, limiting its application in conventional textile production.[18] However, new degumming methods for mulberry fibres have been discovered through recent studies, leading to the development of fibres with better mechanical properties.[20,18] This advancement expands the potential use of mulberry bark fibres in industrial textile applications.[20,18]

Besides the fashion sector, the cosmetic industry is also increasingly focusing on developing sustainable and environmentally friendly materials.[21] In recent years, rising interest in innovative fabrics based on biopolymers for applications such as cosmetic face masks has driven the cosmetic market.[21]

1.2. Fungi-based cosmetic sheet masks

1.2.1. Conventional cosmetic sheet masks and their environmental impact

In recent years, the utilisation of skin care products and the popularity of cosmetic trends has increased, driven by the growing demand of modern society.[22,21] One of the prevalent beauty products in skincare routines, especially in the Asian market, are the cosmetic sheet masks.[22,21] Cosmetic sheet masks are directly applied onto the face and contain a serum with various purposes, such as hydration, anti-ageing effects and brightening.[22,23] Facial sheet masks are woven or non-woven sheets available as dry or wet masks.[22] Unlike wet masks, where the fabric is already soaked in serum, dry sheet masks are packed in the dried state and must be soaked in water or serum before application.[22] Compared to peel-off masks and rinse-off masks, sheet masks are easy and fast to apply and less messy when removing.[23] Conventional facial sheet masks are usually single-use products based on cotton or petroleum-derived fibres.[21,23] Although cotton is a biodegradable and biocompatible raw material, producing cotton fibres requires a considerable amount of land, water and pesticides, making cotton a less ideal sustainable material.[2] In addition, cotton contains impurities like pectin and plant particles, which need to be removed by chemical processes.[2] Cosmetic facial masks based on petroleum-derived fibres are non-biodegradable, usually non-recyclable, and can lead to skin irritation and inflammation.[21] Additionally, the utilisation of fossil resources increases its environmental impact.[21] Furthermore, added emulsifiers, preservatives, and other chemicals of the used serum can also impede biocompatibility.[21] These environmental considerations, together with the continuous expansion of the cosmetic market, underline the necessity for biodegradable facial sheet masks derived from alternative renewable resources.[21]

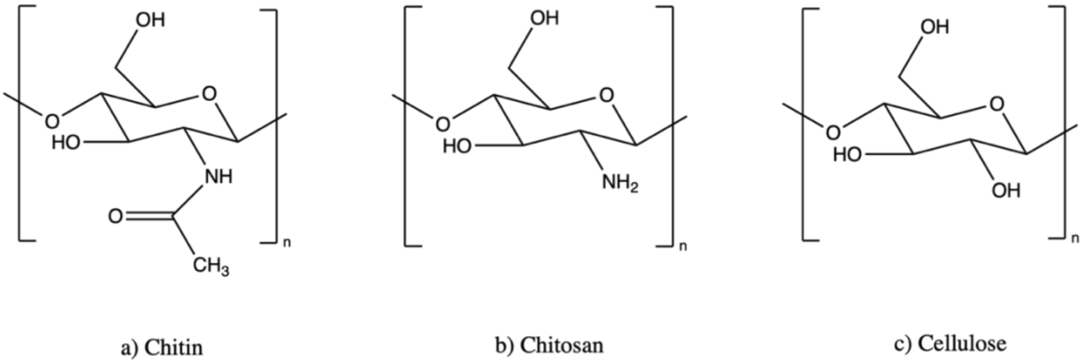
1.2.2. Fungi and their potential use for cosmetic sheet masks

Natural biopolymers are increasingly applied in skin contact products due to being renewable, biodegradable and skin compatible.[24] The most commonly used biopolymers in cosmetics include collagen, bacterial cellulose, chitin, chitosan and lignin.[24] Chitin and chitosan, which show anti-microbial, anti-oxidant and anti-inflammatory properties, have already received significant attention in tissue engineering[24] and thus seem to be a potential material for cosmetic sheet masks.

While commercial sheet masks usually obtain their main cosmetic compounds from the added serum, fungal biomass itself carries valuable components beneficial for the skin.[22,25] Numerous fungi species were found to produce compounds with high potential in cosmetical skin care formulations, such as anti-ageing, moisturising, and anti-oxidative properties.[25,26] Only a few of many examples are chitin-glucan co-polymers, polysaccharides, carotenoids, phenolic compounds and vitamins, which are found in many fungi species and already used in cosmetical products.[25,27] Chitin-glucans are, for instance, known to possess skin moisturising and anti-ageing properties and are thus used in many cosmetical formulations.[26] Other polysaccharides, such as α and β-glucans, can increase the skin's moisture retention capacity and prevent moisture release.[27] Carotenoids show anti-inflammatory, free radical scavenging and UV protection activities.[27] Phenolic compounds, such as caffeic or gallic acid, are known for their anti-oxidative potential and help brighten the skin through melanin reduction.[27,28] In this study, the focus lies on the mushroom types white button mushrooms (*Agaricus bisporus*), Shiitake (*Lentinula edodes*) and Lion's mane mushrooms (*Hericium erinaceus*). These species contain manifold compounds potentially beneficial to the skin, including the antioxidants L-ergothionein and trehalose, vitamin D2 and B12 and many more.[25,27] Fungi-derived products contain valuable cosmetical compounds[25,27] and improve economic circularity in the cosmetic industry through their biocompatibility and renewability.[21] Thus, fungi-based cosmetical sheet masks represent an interesting opportunity for the innovation of cosmetic products.

1.3. The origin of chitin, chitosan and β-glucan

Chitin is the second most abundant biopolymer on earth after cellulose.[4,29] This white fibrous biopolymer comprises N-acetyl-d-glucosamine units linked by (1,4)-β-glycosidic bonds.[4,29] Chitin is a rigid polysaccharide and is the structural key component in the cell walls of fungi and the main constituent of the exoskeletons of arthropods, such as insects and crustaceans.[30,31] Its main derivative, chitosan, is obtained by deacetylation of chitin and shows an increased solubility due to the amine group.[30,31] The molecular structures of chitin and chitosan are highly similar to cellulose (Figure 1), only differing in the C2 position, where the hydroxyl group is replaced with an acetamide (chitin) or amine group (chitosan).[32]



a) Chitin b) Chitosan c) Cellulose

Figure 1: Chemical structures of (a) chitin, (b) chitosan and (c) cellulose.

Due to their non-toxicity, adsorption properties and biocompatibility, chitin and chitosan are widely used in various applications, particularly in the medical field for wound treatment or drug delivery, as well as in the cosmetic and food industry.[30,31] Furthermore, the high nitrogen content is the reason for its extensive use as a chelating agent for dye and heavy metal ions in wastewater treatment.[31] Chitin is mainly extracted from crustacean shells and marine waste. However, procurement from marine sources is not only associated with ethical conflicts but is also highly dependent on seasonal availability and fluctuations.[30,32] In contrast, the extraction of chitin from fungi offers several advantages, such as easy accessibility to the biopolymer in the cell wall, lower operational costs, and less need for chemicals and energy during extraction.[30] Unlike crustacean chitin, fungi-derived chitin is free from animal-regarded ethical issues and does not contain crustacean allergic proteins, allowing increased usability.[30,32] Chitin appears in the fungal cell wall as nanofibrils[29] and mainly derives its strength from the hydrogen bonds between the macromolecular chains.[3] Unlike crustacean chitin, fungi-derived chitin is covalently linked to β-glucan (Figure 2), another structural element of the fungal cell wall comprising glucose polymers mainly linked by linear (1,3)-β-glycosidic bonds.[4,30]

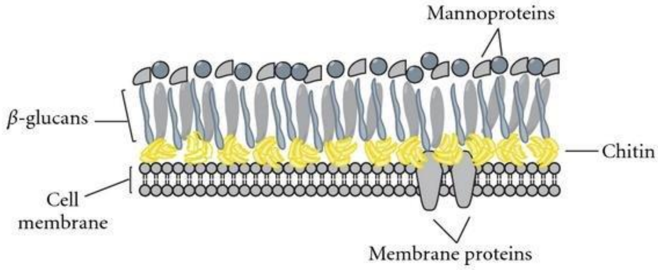


Figure 2: Structure of fungal cell walls, consisting of chitin, covalently bound β-glucan and other membrane proteins[33].

The proportions of chitin and β-glucan vary depending on the fungi species and cultivation conditions.[30] The covalent linkage of both biopolymers constitutes a natural composite, combining the strength of chitin and the flexibility and toughness of β-glucan.[29] Furthermore, this chitin-β-glucan complex (CG complex) shows increased film-forming properties and provides different surface characteristics.[29]

The CG complex can be obtained from fungal fruiting bodies through mild extraction steps.[29,34] Afterwards, it can be resuspended and filtrated for the production of chitin nanopapers, as illustrated in Figure 3.[29,34] For the extraction of the chitin-glucan complex, the mushrooms are rinsed with water to remove dirt and blended. Afterwards, the suspension is heated in distilled water to remove water-soluble components. For deproteination and to remove lipids and alkali-soluble components, the suspension is stirred in 1M NaOH.[29,34] To prepare chitin nanopapers, the extracted CG complex is resuspended and blended with water and vacuum filtrated. The resulting filter cake is pressed on a heating plate between blotting papers to remove excess water.[29] Nawawi et al.[35] reported tensile strength of more than 200 MPa and moduli of 7 GPa for chitin nanopapers prepared using the process described above. As chitin is brittle in its raw form, plasticisers such as glycerol can be added to increase flexibility and elasticity.[31,36] Chitin- and β-glucan based materials are rapidly attracting attention as a green alternative replacing synthetic packaging and foams or conventional textiles, such as bovine and artificial leather.[4]

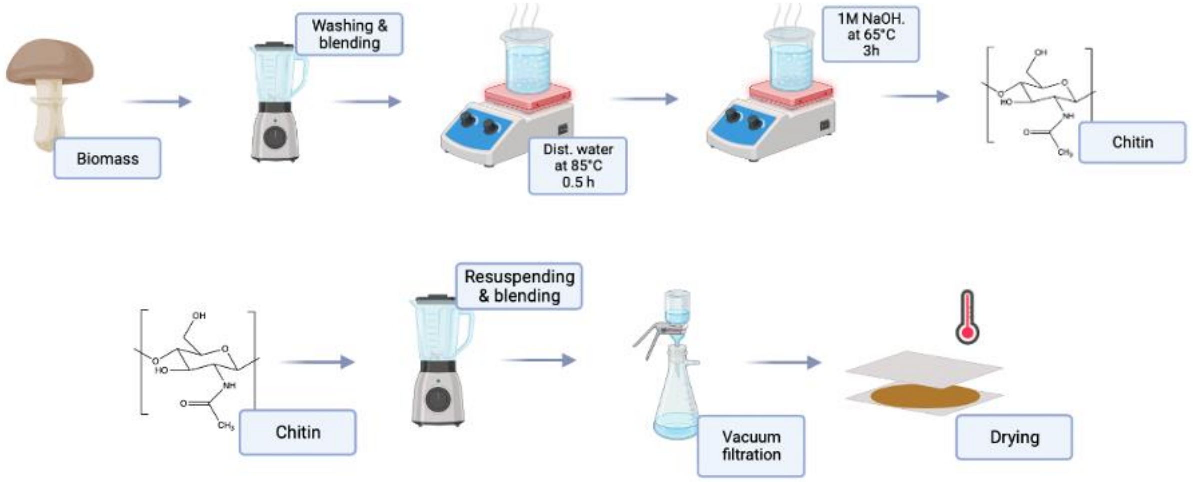


Figure 3: Process of the extraction of the chitin-glucan complex from fungi and nano-paper preparation (Created with BioRender.com)

2. Aim and Objectives

This project aimed to develop and characterise textile materials derived from fungal fruiting bodies for applications such as leather substitutes and cosmetic sheet masks. A suitable extraction method of the chitin-glucan complex from various fungi had to be established for each application. The preparation pathway to produce cosmetic masks and leather-like materials was optimised. Reinforcement methods with fibres were developed to improve the mechanical properties of fungi-based leather substitutes. Finally, the produced sheets were characterised with regards to their porosity, mechanical and surface properties and moisture content.

3. Experimental

3.1. Materials

White button mushrooms (*Agaricus bisporus*) were purchased in local stores in Vienna. Shiitake (*Lentinula edodes*) and Lion's mane mushrooms (*Hericium erinaceus*) were cultivated and kindly provided in a frozen state by the Institute of Material Chemistry of the University of Vienna. Ethanol was purchased from Brenntag Austria GmbH. Sodium Hydroxide (NaOH) and glycerol were bought from W. Neuber's Enkel Groß-Drogerie, Austria. Lyocell fibres were kindly provided by Lenzing AG, Austria, and mulberry bark fibres (white) were purchased from Eva Gora Papierkunst, Germany. Synthetic leather made of polyurethane and cotton of the brand Generic was bought over Amazon from Shenzhen xing hui teng plastic co., LTD, China. Smooth bovine leather blanks from CLUB4BRANDS were purchased over Amazon from JMFA LTD, UK. Compressed one-way facial masks made from cotton were purchased over Amazon from Akissco-de. The coating material OC-BioBinder^{TM} Lotus 54XX was bought from OrganoClick, Sweden.

3.2. Development of fungi-based leather-like materials

3.2.1. Extraction of the chitin-glucan complex

500 g of white button mushrooms were washed and blended for 3 min in 500 ml distilled water using a blender (Vita Power 2000). Afterwards, the suspension was heated in 2 L of 0.1 M NaOH at 65 °C for 0.5 h to remove lipids, water-soluble and alkali-soluble components.

Compared to the procedure previously described in Chapter 1.1, the milder extraction method was used to obtain a softer surface of the resulting CG sheet. After cooling down, the suspension was vacuum filtrated through a kitchen towel in a Büchner Funnel and neutralised with distilled water. The dry mass (DM) of the resulting chitin-glucan complex was determined. For this, three samples with around 0.5 g of CG complex were weight in a wet state (m_w) and again after drying in an oven at 70 °C overnight (m_d) (Eq. 1). The average of the three samples was taken for the calculation of the dry mass.

$$DM\ [\%] = \frac{m_d}{m_w} \cdot 100 \qquad \text{(Eq. 1)}$$

3.2.2. Chitin-glucan sheet preparation

Using the calculated dry mass, the amount of the CG complex for a sheet with a grammage of 100 g/m^2 was determined. The amount of biopolymer was blended for 1 min. with 100 ml dist. H_2O and 30 ml glycerol. In the previous work by Julia Jüstel[37], this amount of plasticiser was found to be optimal for achieving flexible and foldable chitin-glucan sheets. A filter paper was placed in a filter funnel and moistened with water. Afterwards, the funnel was levelled with a water balance to achieve an even distribution of the suspension and a uniform film thickness. The suspension was vacuum filtered and dried between two metal plates with metal meshes, blotting paper and a weight of 5 kg on top at 50 °C on a heating plate for 48 h.

3.2.3. Reinforcement with fibres

The chitin-glucan sheets were reinforced with lyocell and mulberry fibres to improve mechanical properties.

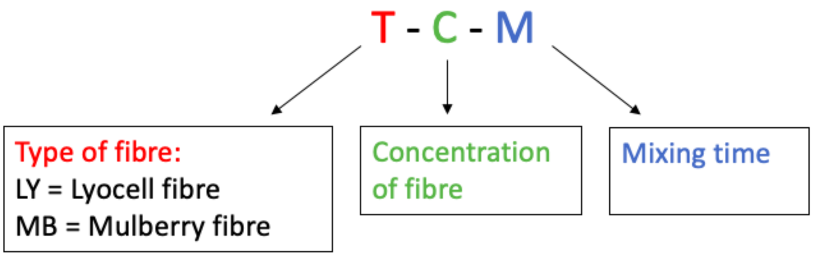
3.2.3.1. Lyocell fibres as reinforcement for leather-like materials

10 wt.% (of DM) of lyocell fibres with a length of around 3-4 cm were finely pulled apart by hand and suspended in a beaker with 200 ml Ethanol. Ethanol was used as a solvent to decrease the aggregation of the lyocell fibres. Using the previously calculated dry mass, the amount of CG for a sheet with 100 gsm was blended for 1 min with 100 ml Ethanol (EtOH) and 30 ml glycerol. The CG suspension was manually mixed with the lyocell fibres and filtered and

dried as described in Chapter 3.2.2. The reinforcement of CG sheets with lyocell fibres will be labelled “LY”.

3.2.3.2. Mulberry fibres as reinforcement for leather-like materials

The mulberry fibres were soaked in dist. water for 24 h. CG sheets of 100 gsm were prepared by blending the respective amount of chitin-glucan with 100 ml dist. H_2O, 30 ml glycerol and soaked mulberry fibres. Different mass fractions of 10, 20, 30 and 40 wt.% with regard to the dry mass of CG were used. Furthermore, different blending times of 1, 2, 4, 6 and 8 min were tested for CG sheets with 40 wt.% of mulberry fibres. The suspension was filtered and dried as previously described. The reinforcement of CG sheets with mulberry fibres will be labelled “MB”. The samples will be labelled as follows:



3.2.4. Coating of leather-like materials

The reinforced CG sheets were coated with a biobased hydrophobic binder to improve surface properties and increase hydrophobicity. The coating required 12 g/m^2 of OC-BioBinder^{TM} Lotus 54XX. For this, 0.07 g OC-BioBinder was diluted with 0.18 g dist. water and manually distributed on a CG sheet of 100 gsm with mulberry fibre reinforcement (MB-40%-4min). The sheet was dried between baking paper, blotting paper and two metal plates with 5 kg on top at 50 °C for 24 h.

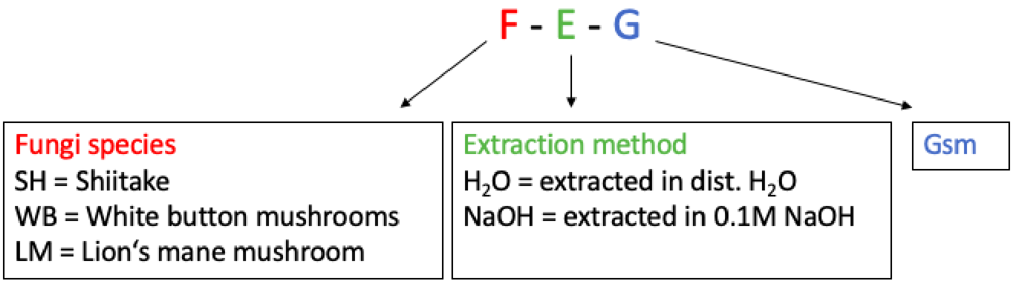
3.3. Development of fungal cosmetic masks

3.3.1. Extraction of chitin-glucan complex

For the development of fungal cosmetic masks, three different types of fungi were tested: white button mushrooms (*Agaricus bisporus*), Shiitake (*Lentinula edodes*) and Lion’s mane mushrooms (*Hericium erinaceus*). 500 g of fruiting bodies were washed and blended for 3 min. in 500 ml distilled water using a kitchen blender (Vita Power 2000). For the development of fungal cosmetic masks, two extraction methods were tested. In the first extraction method, the suspension was heated in 2 L 0.1 M NaOH at 65 °C for 0.5 h. After cooling down, the suspension was vacuum filtrated through a kitchen towel in a Büchner Funnel and neutralised with distilled water. In the second extraction method, the suspension was heated at 2 L dist. H_2O at 65° C for 0.5 h. This was done to preserve as many valuable cosmetic components of the fungi in the final cosmetic masks. The extraction method using 0.1 M NaOH will be labelled "NaOH", and the method using dist. water will be labelled "H_2O". The dry mass of the obtained CG complex was determined equally as in Chapter 3.2.1.

3.3.2. Chitin-glucan sheet preparation for cosmetic masks

Chitin-glucan films with 50 and 100 g/m² were prepared for fungal cosmetic masks. The respective amount of biopolymer was blended for 1 min with 100 ml dist. H_2O and 30 ml glycerol and filtered through a filter funnel with a previously moistened filter paper. The sheets were dried between two metal plates with metal meshes and blotting paper and a weight of 5 kg on top at 50 °C on a heating plate. CG sheets with 50 gsm were dried for 24 h and 48 h for sheets with 100 gsm. The samples will be labelled as follows:


F - E - G

Fungi species
SH = Shiitake
WB = White button mushrooms
LM = Lion's mane mushroom

Extraction method
H_2O = extracted in dist. H_2O
NaOH = extracted in 0.1M NaOH

Gsm

3.4. Characterisation methods

3.4.1. Determination of porosity of fungi-based leather substitutes and cosmetic masks

The porosity (Φ) of the prepared CG sheets was calculated using the envelope (ρ_e) and skeletal density (ρ_s). For the envelope density, the mass (m) of each specimen was divided through the area (A) and the thickness (d). The skeletal density was measured by gas displacement with Helium using the Micromeritics AccuPyc II 1340 with a chamber of 1 cm^3. The CG sheets were cut into around 3x3 mm pieces and dried overnight in an oven at 80 °C before measurement. Each sheet was measured in 10 replicates. The following equations were used for the calculations:

$$\rho_e\ [kg \cdot m^{-3}] = \frac{m}{A \cdot d} \qquad \text{(Eq. 2)}$$

$$\Phi\ [\%] = \left(1 - \frac{\rho_e}{\rho_s}\right) \cdot 100 \qquad \text{(Eq. 3)}$$

3.4.2. Mechanical properties of fungi-based leather substitutes and cosmetic masks

To characterise the mechanical properties, the prepared sheets were cut (Zwick ZCP 020 Manual Cutting Press, Zwick, Ulm, Germany) into dog bone-shaped specimens with a parallel width of 5 mm and an overall length of 75 mm (Type 1BA, EN ISO 527-2). The weight was determined using a microbalance (Sartorius Cubis, Sartorius, Göttingen, Germany) to calculate the grammage of each specimen. The thickness was determined with an outside micrometre gauge (Digital Outside Micrometre 0-25 mm, AnyiMeasuring, Guilin City, P.R. China) at four random locations at the parallel section of the specimen. The tensile strength, strain to failure and Young's modulus were determined by tensile tests using an Instron universal testframe (Model 5969 Column Universal Testing System, Instron, Darmstadt, Germany) with a load cell of 1 kN and a testing velocity of 1 mm/min. The specimens were fixed between metal clamps with two layers of blotting paper in between to avoid damage to the specimen. The Young's modulus (E) was calculated by determining the slope of the linear elastic region separated by at least 0.2 % strain of the strength value. The tensile strength (σ) is determined by dividing the maximum load by the cross-sectional area of the specimen. A threshold of 0.1 MPa was chosen for the calculations. The corresponding engineering strain was defined as the strain to failure. For each sheet, a minimum of 5 to 8 specimens were tested.

3.4.3. Water contact angle of fungi-based leather-like materials

The water contact angle was measured to determine the hydrophobicity before and after the coating process. This was done using the Krüss DSA30 drop shape analyser with a dose volume of 5 μl and a dosing rate of 0.16 μl/s. The contact angle was determined with a delay of 5, 30, 60 and 120 s and was performed with 30 replicate measurements. For each measurement, the average of both contact angles was calculated. The contact angle was measured for the coated and uncoated MB-40%-4min, bovine leather and artificial leather made from PU. To determine significant differentiations of the mean values after an exposure time of 5 s, statistical analysis was performed via analysis of variance (ANOVA), followed by Tukey post-hoc.

3.4.4. Determination of mulberry fibre length

To determine the fibre length after various blending times, mulberry fibres were soaked for 24 h. Fine pieces of mulberry fibre were blended with 400 ml of dist. water for 1, 2, 4, 6 and 8 min. Afterwards, the suspension was filtered in a Büchner funnel with a black silk paper as filte. The black silk paper was dried for 1 h at RT and scanned with a printer from image RUNNER advance from Canon. The resulting images were analysed using ImageJ to measure the fibre length. For each blending time, a minimum of 100 fibres were measured. Statistical analysis was performed via ANOVA, followed by Tukey post-hoc.

3.4.5. Moisture content of fungal cosmetic masks

Prior to application, the fungal cosmetic masks should be soaked in water for 3 min. To determine the moisture content afterwards, the CG sheets were analysed gravimetrically using a microbalance (Sartorius Cubis, Sartorius, Göttingen, Germany). The CG sheet was cut into around 5x8 mm pieces and weighed before and after soaking in water for 3 min and again after 5, 10, 15 and 20 min. Each sheet was measured in triplicates at a 21-23.2 °C temperature range and relative humidity of 21-32 %. The moisture content was calculated with the ratio of the mass of retained liquid (m_l) to the mass of the dry textile (m_t) (Eq. 4). Significant differences between each extraction method for the same fungi type were determined using ANOVA, followed by Tukey post-hoc .

$$Moisture\ content\ [\%] = \frac{m_l}{m_t} \cdot 100 \qquad \text{(Eq. 4)}$$

4. Results and Discussion

4.1. Fungi-derived leather-like materials

4.1.1. Reinforcement with lyocell

The reinforcement with lyocell fibres resulted in an inhomogeneous distribution of fibres within the CG sheet (Figure 4). The reason for this could be the aggregation of the lyocell fibrils in solution.[38] In addition, the low stiffness of the fibres made them difficult to blend and homogenise with a mixer. The reinforced sheets showed high flexibility and were foldable. However, due to the non-uniform distribution of lyocell fibres, the smoothness also differed extremely within the sheet. Areas with high concentrations of lyocell felt more rough than areas with lower amounts, which felt softer and smoother.

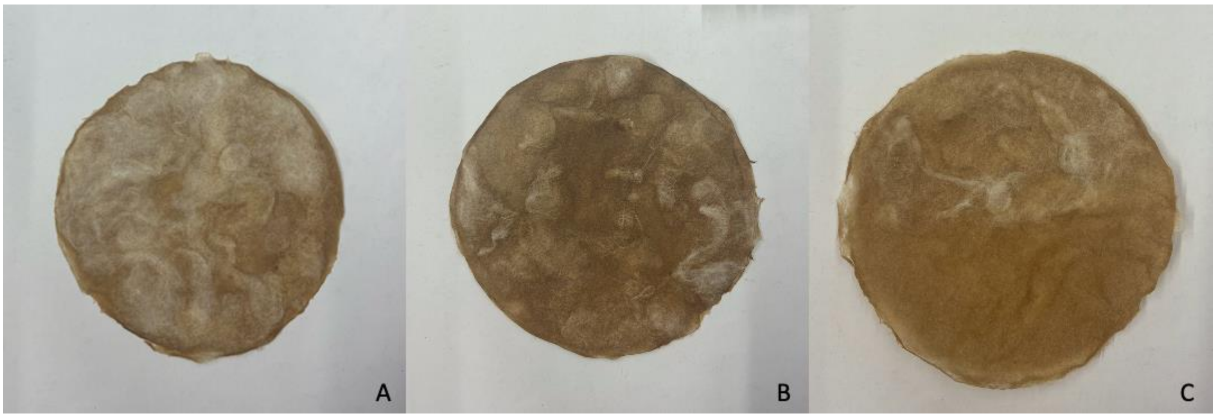


Figure 4: A-C show CG sheets with 100 gsm and reinforced with lyocell fibre.

4.1.2. Reinforcement with mulberry fibres with varying mass fractions

Due to their stiffness, the mulberry fibres could be blended easily and distributed more homogeneously within the CG sheet than lyocell fibres. Figure 5 shows the resulting sheets with varying mass fractions of 10, 20, 30 and 40 wt.% of mulberry fibre after a blending time of 4 min. All mass fractions resulted in flexible, soft and foldable sheets. With increasing mulberry fibre content, the surfaces were slightly rougher. Due to the higher homogeneity and the smoother texture compared to sheets with lyocell fibres, the mulberry fibres were selected as a more suitable reinforcement material for fungi-based leather-like materials.

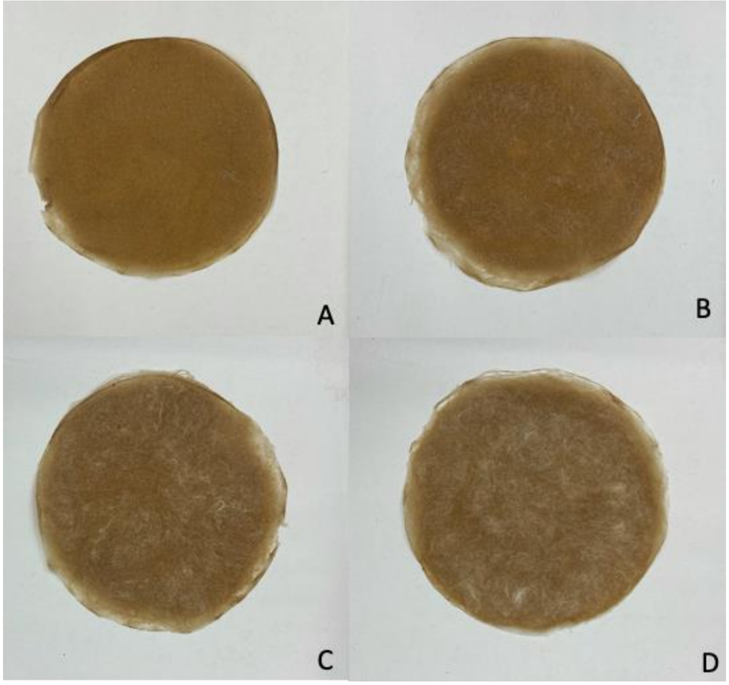


Figure 5: CG sheets with 100 gsm reinforced with mulberry fibre with mass fractions of 10 wt.% (A), 20 wt.% (b), 30 wt.% (C) and 40 wt.% (D).

4.1.2.1. Characterisation of mulberry reinforced CG sheets

The produced CG sheets, the bovine and PU leather were characterised by grammage (gsm) and thickness (d). The results are shown in Table 1. A grammage of 100 gsm was targeted, which was exceeded due to the addition of glycerol and the mulberry fibres. This is because the weight depends on the retained amount of glycerol during the vacuum filtration step, which varies from sheet to sheet depending on the filtering time and strength of the vacuum applied.

Table 1: Grammage (gsm) and thickness (d) of the produced sheets reinforced with varying concentrations of mulberry fibres and references

Sample	**gsm [g/m²]**	**d [μm]**
Pure CG with glycerol	140.7 ± 5.6	141.3 ± 7.6
MB-10%-4min	218.9 ± 8.1	197 ± 12
MB-20%-4min	253.6 ± 11.2	235 ± 6
MB-30%-4min	249.7 ± 16.0	246 ± 15
MB-40%-4min	240.8 ± 4.7	249 ± 10.3
PU leather	350.5 ± 2.4	650.7 ± 4.7
Bovine leather	791.0 ± 8.0	1085.0 ± 25.1

The high standard deviations of the grammage and thickness are due to inhomogeneities in the distribution of mulberry fibres. The thickness of the sheets increased with higher mulberry fibre content, which is caused by the additional volume and the created voids between fibres. Furthermore, the envelope density, skeletal density and porosity were determined and are summarised in Table 2. The porosity is an essential aspect since it affects the softness and the ventilating properties of leather.[39,40] A high degree of breathability and air permeability are crucial for a comfortable wearing sensation.[39,40,41] While the envelope density slightly decreased with fibre content, the skeletal density remained stable. This resulted in higher porosity with increasing mulberry fibre content, which can be explained by the higher number of voids between mulberry fibres reaching a porosity of 30 % by MB-40%-4min. Compared to the reinforced CG sheets, PU and bovine leather showed higher porosities. PU leather showed the highest porosity of 67 %, followed by bovine leather, with a porosity of 48 %. The high porosity of the tested synthetic leather could derive from the backside, which is made of woven cotton. The usual porosity of bovine leather is reported by Xiu He et al. to be between 58-63 %.[41] The typical porosity of pure chitin-glucan sheets is around 67 % with a skeletal density of 1.40 g/cm^3.[29] By adding glycerol, the porosity of chitin-glucan sheets can decrease to 13 %, which is caused by the ability of glycerol to intercalate in small voids between chitin nanofibers.[42] The mass fraction of 40 wt.% mulberry fibres was chosen as a suitable concentration for further sheet production due to the high porosity. A higher mass fraction could increase the porosity, but the surface would get rougher and stiffer, affecting the leather-like haptic.

Table 2: Envelope density (ρ_e), skeletal density (ρ_s) and porosity (Φ) of the produced sheets and references.

Sample	**ρ_e [g/cm^3]**	**ρ_s [g/cm^3]**	**Φ [%]**
Pure CG with glycerol	1.00 ± 0.03	1.15 ± 0.015	13.1 ± 2.7
MB-10%-4min	1.11 ± 0.05	1.35 ± 0.001	17.7 ± 4.1
MB-20%-4min	1.08 ± 0.04	1.36 ± 0.002	20.3 ± 3.2
MB-30%-4min	1.02 ± 0.04	1.36 ± 0.003	25.1 ± 2.7
MB-40%-4min	0.97 ± 0.04	1.39 ± 0.003	30.3 ± 2.6
PU leather	0.54 ± 0.01	1.62 ± 0.010	66.8 ± 0.5
Bovine leather	0.73 ± 0.01	1.40 ± 0.003	47.7 ± 1.0

4.1.2.2. Mechanical properties of mulberry reinforced CG sheets

To compare the mechanical properties of the reinforced CG sheets, the tensile strength (σ), Young's modulus (E) and strain to failure (ε) were determined (Table 3). Figure 6 shows the representative stress-strain curves of the CG sheets with varying amounts of mulberry fibre. The pure CG sheet with glycerol shows the lowest tensile strength and modulus. After adding mulberry fibre, both values increased significantly, emphasising successful reinforcement. The improved tensile strength of the reinforced sheets is due to the physical and chemical interactions such as fibril entanglement, space-filling and surface adhesion between mulberry fibres and the chitin-glucan matrix.[43] The tensile strength and modulus, thus stiffness, increases with higher mulberry content than 20 wt.%. The CG sheet MB-40%-4min showed the highest tensile strength of 9.9 MPa and a modulus of 113.5 MPa. This tensile strength is comparable to that of mycelium-based leather alternatives and PU leather, which typically have tensile strength ranging from 5.6-12.5 MPa and up to 13 MPa.[12,14] As reported by Harnagea[13], the tensile strength of bovine leather usually falls between 7-22 MPa, while the tested bovine leather showed a tensile strength of 7.2 MPa, comparable with MB-30%-4min. The moduli of the mulberry reinforced sheets are comparable to or even exceed the reported moduli for bovine leather, which typically is between 20-100 MPa.[13,44]

Table 3: Tensile strength (σ), Young's modulus (E) and strain to failure (ε) of the produced sheets with varying amounts of mulberry fibre.

Sample	**σ [MPa]**	**E [MPa]**	**ε [%]**
Pure CG with glycerol	3.2 ± 0.4	53.0 ± 12.1	12.2 ± 0.7
MB-10%-4min	7.5 ± 0.9	82.0 ± 14.8	15.3 ± 1.5
MB-20%-4min	6.0 ± 0.7	65.5 ± 8.9	11.3 ± 1.4
MB-30%-4min	7.8 ± 1.3	83.5 ± 12.9	11.0 ± 1.2
MB-40%-4min	9.9 ± 0.8	113.5 ± 17.6	10.4 ± 1.0
Bovine leather	7.2 ± 0.7	9.9 ± 1.6	44.0 ± 5.6

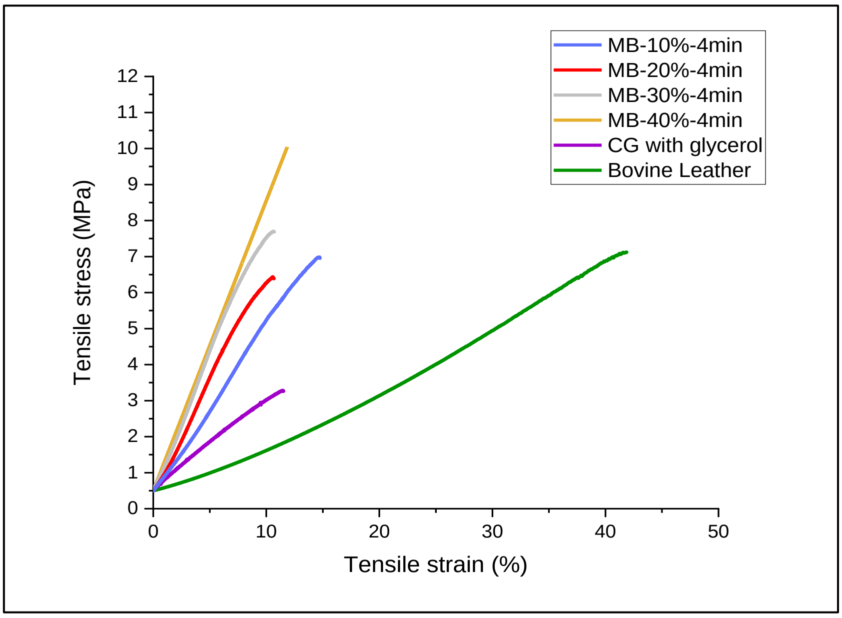


Figure 6: Representative stress-strain curves of produced CG sheets reinforced with varying amounts of mulberry fibre and bovine leather.

Among the produced sheets, MB-10%-4min, with the lowest fibre content, achieved the highest tensile strain of 15.3 %. This could be due to the higher volume fraction of glycerol in the sheets with less fibre content. Glycerol intercalates and interacts with the polymers via hydrogen bond formation. This increases intramolecular space between the polymers and polymer mobility, leading to higher tensile strain.[36,42] When increasing the fraction of mulberry fibre to 20 wt.% or higher, the strain to failure drops to around 10-11 %. The reason for this could be that the stiffness of the mulberry fibre dominates at higher fractions, therefore reducing the tensile strain. The tensile strain of the tested bovine leather peaked at a value of 44.0 %, which is around four times higher than the produced CG sheets. The tensile strain of bovine leather is reported to be usually between 35-56 %, while PU leather ranges from 15-60 %.[13,14,3] Mycelium-based leather imitates are reported to show tensile strains at 16-80 %.[12]

Figure 7 shows the rupture location after the tensile tests of the mulberry fibre-reinforced sheets (A-D) and bovine leather (E). At the rupture of the CG sheet with the lowest mass fraction of 10 wt.% (A) only very few fibres can be observed that are pulled out by mechanical forces. In comparison, samples with higher fibre content (B-D) have more exposed fibres, miming the rupture point of bovine leather with exposed collagen fibrils (E).

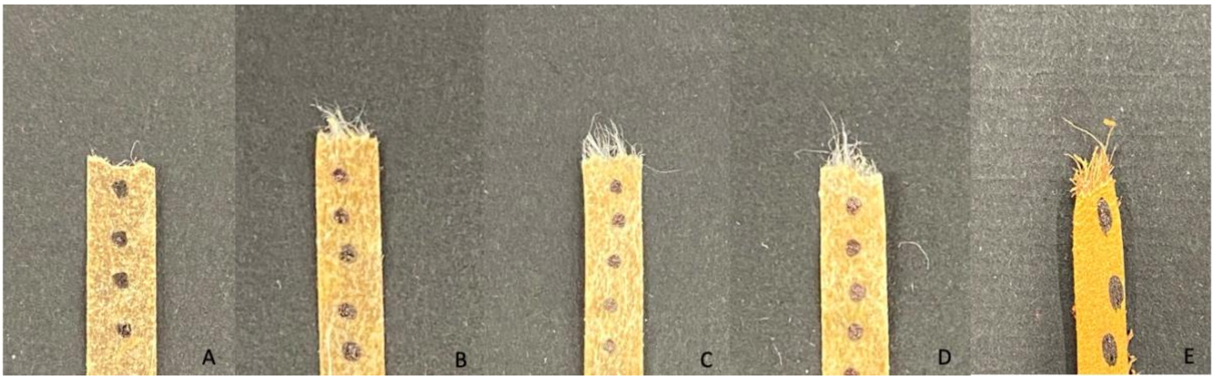


Figure 7: Rupture of the reinforced CG sheets with mulberry fibre mass fractions of 10 wt.% (A), 20 wt.% (B), 30 wt.% (C), 40 wt.% (D) and bovine leather (E) after tensile tests.

4.1.3. Reinforcement with mulberry fibres with varying blending times

The following chapter investigated if different blending times influence the length of mulberry fibres and the mechanical properties of the reinforced sheets. Soaked mulberry fibres (40 wt.%) with an original length of around 6 mm were blended for 1, 2, 4, 6 and 8 min before sheet preparation.

4.1.3.1. Characterisation of mulberry reinforced CG sheets with varying blending times

The grammage (gsm), the thickness (d) of the produced sheets, and the fibre length (l) after the respective mixing times were determined. Table 4 shows that the grammage and thickness of the produced CG sheets varied between each sheet and showed high standard deviations.

Table 4: Grammage (gsm), thickness (d) and fibre length [l] of the produced sheets reinforced with 40 wt.% of mulberry fibres with varying blending times

Sample	**gsm [g/m^2]**	**d [μm]**	**l [mm]**
MB-40%-1min	228.2 $\pm$ 5.7	229.1 $\pm$ 15.9	5.9 $\pm$ 0.9
MB-40%-2min	209.3 $\pm$ 11.1	211.3 $\pm$ 9.9	6.0 $\pm$ 1.2
MB-40%-4min	240.8 $\pm$ 4.7	249 $\pm$ 7	5.8 $\pm$ 1.4
MB-40%-6min	256.3 $\pm$ 10.2	230.3 $\pm$ 12.0	5.6 $\pm$ 1.5
MB-40%-8min	237.2 $\pm$ 9.0	212.8 $\pm$ 16.1	5.2 $\pm$ 1.3

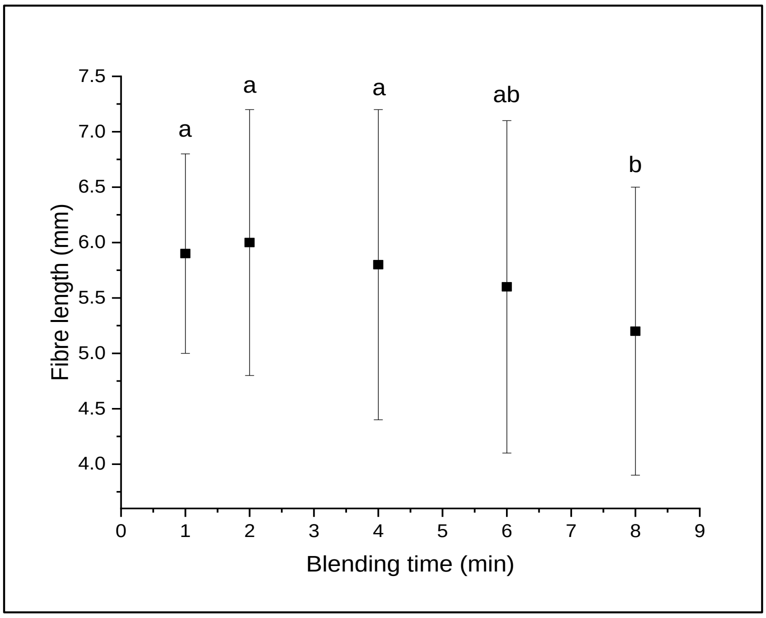


Figure 8: Comparison of the mean fibre length of the blended mulberry fibre with standard deviation after 1, 2, 4, 6 and 8 minutes of blending time. ANOVA test and Tukey post-hoc analysis were performed to determine significant differentiation. Mean values with the same letter did not show significant differences. Only the mean value of 8 minutes of blending time showed a significant difference from the values of 1, 2 and 4 minutes ($p < 0.05$).

Again, the reasons for this were the different retentions of glycerol during filtration and inhomogeneities in the distribution of mulberry fibres (see Chapter 4.1.2.1). As illustrated in Figure 8, the fibre length decreased slightly after 4, 6 and 8 min. In addition, the deviation was higher for fibres after longer blending times. However, only the mean fibre length after 8 min of blending time differed significantly from the values after 1, 2 and 4 min, as concluded with ANOVA and Tukey post-hoc analysis.

Furthermore, the envelope density, skeletal density and porosity were determined and are shown in Table 5. The reinforced CG sheets MB-40%-6min and MB-40%-8min show lower porosities than those with shorter blending times of 1, 2 and 4 min. This could be explained by the slightly shorter fibres after 8 min of blending and the high standard deviations after 6 min of blending, indicating a more heterogenous fibre length distribution. Shorter fibres can fill the voids between the larger mulberry fibres and chitin nanofibers, thus reducing the porosity.

Table 5: Envelope density (ρ_e), skeletal density (ρ_s) and porosity (Φ) of the produced sheets.

Sample	ρ_e **[g/cm³]**	ρ_s **[g/cm³]**	Φ **[%]**
Pure CG with glycerol	1.00 ± 0.03	1.15 ± 0.015	13.1 ± 2.7
MB-40%-1min	1.00 ± 0.05	1.39 ± 0.003	28.5 ± 3.4
MB-40%-2min	0.99 ± 0.04	1.41 ± 0.001	29.9 ± 3.0
MB-40%-4min	0.97 ± 0.04	1.39 ± 0.003	30.3 ± 2.6
MB-40%-6min	1.12 ± 0.04	1.36 ± 0.0002	17.4 ± 3.2
MB-40%-8min	1.11 ± 0.05	1.38 ± 0.003	19.7 ± 3.5
PU leather	0.54 ± 0.01	1.62 ± 0.010	66.8 ± 0.5
Bovine leather	0.73 ± 0.01	1.40 ± 0.003	47.7 ± 1.0

4.1.3.2. Mechanical properties of mulberry reinforced CG sheets with varying blending times

To determine the influence of blending time and fibre length on the mechanical strength, the tensile strength (σ), strain to failure (ε) and modulus (E) were measured. Table 6 shows the summarised results, and Figure 9 illustrates the representative stress-strain curves of the produced sheets and of bovine leather. The results show that the strain to failure stays stable around 10-12 % for all samples, except for the tested bovine leather, which achieved a max. tensile strain of 44 %. On the contrary, the tensile strength and modulus differ with varying blending times. MB-40%-2min reached the highest tensile strength and modulus with 19.6 MPa and 612.7 MPa, followed by MB-40%-1min with a tensile strength of 14.1 MPa and a modulus of 286.1 MPa. This correlates with the mulberry fibre length discussed in Chapter 4.1.3.1, where a blending time of 1 and 2 min also resulted in the longest mulberry fibre length. The achieved tensile strength of MB-40%-2min and MB-40%-1min exceeded the values of the tested bovine leather with 7.2 MPa. The obtained values were also higher than reported tensile strength for mycelium-based and PU leather, which are usually between 5.6-12.5 MPa and up to 13 MPa.[12,14] The tensile strength reported for bovine leather typically ranges from 7-22 MPa[13].

In comparison to the sheets with a blending time of 1 and 2 min, the tensile strength and modulus are decreased for sheets with longer blending times. This shows that the blending time affects the mechanical properties in terms of tensile stress and modulus, even if the fibre length only differs slightly. Furthermore, all reinforced CG sheets exceeded the tested bovine leather's modulus, which only had a modulus of 9.9 MPa. In the literature, the moduli for bovine leather are commonly reported to fall between 20-100 MPa.[13,44]

Table 6: Tensile strength (σ), modulus (E) and strain to failure (ε) of the produced sheets and references.

Sample	σ [MPa]	E [MPa]	ε [%]
Pure CG with glycerol	3.2 ± 0.4	53.0 ± 12.1	12.2 ± 0.7
MB-40%-1min	14.1 ± 1.5	286.1 ± 40.0	11.4 ± 1.0
MB-40%-2min	19.6 ± 2.1	612.7 ± 73.8	10.0 ± 0.7
MB-40%-4min	9.9 ± 0.8	113.5 ± 17.6	10.4 ± 1.0
MB-40%-6min	11.0 ± 2.0	160.4 ± 36.5	11.8 ± 0.9
MB-40%-8min	11.0 ± 1.2	157.3 ± 23.2	11.8 ± 0.9
Bovine leather	7.2 ± 0.7	9.9 ± 1.6	44.0 ± 5.6

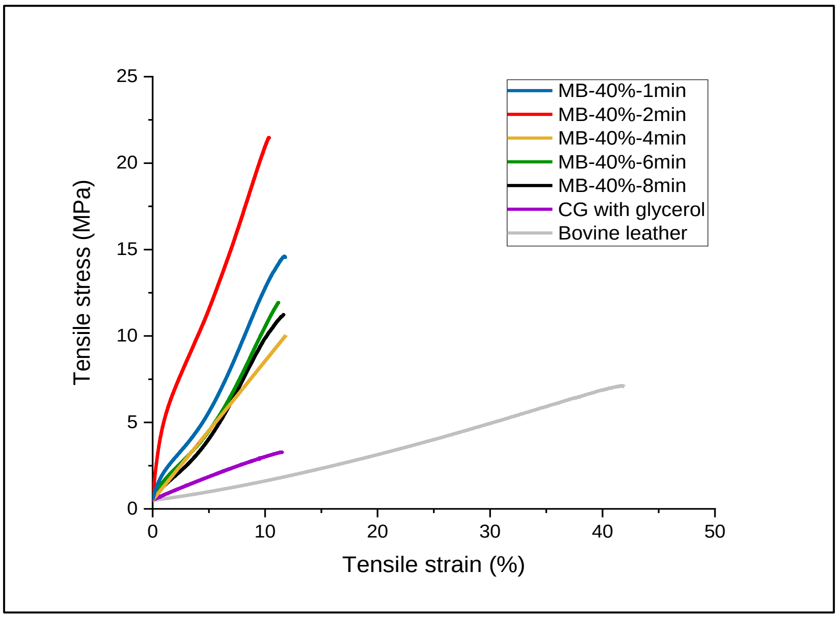


Figure 9: Representative stress-strain curves of produced CG sheets reinforced with 40 wt.% mulberry fibre after different blending times and bovine leather.

4.1.4. Water contact angle of mulberry fibre reinforced CG sheets

To improve surface properties, a CG sheet reinforced with mulberry fibre was coated with a hydrophobic OC-BioBinder^{TM}. To confirm the improved hydrophobicity, the water contact angles were measured. The measurement was taken after an exposure time of 5, 30, 60 and 120 s for a pure CG sheet with glycerol, for a MB-40%-4min sheet with and without coating and the PU and bovine leather materials. The contact angles and the decrease after the total exposure time are summarised in Table 7. The mean water contact angles after an exposure time of 5 s are illustrated in Figure 10. The CG sheet with glycerol showed a contact angle of 85° after 5 s and a decrease of 3 % after 2 min.

In comparison, the contact angle of pure mycelium sheets without glycerol is reported to be 129°.[36] This is because the glycerol treatment reduces the water contact angle due to its hydrophilicity. With reinforced mulberry fibres, the contact angle further decreases to 71° after 5 s and has a high decrease of 19 % after 2 min. The water contact angle decreased due to the hydrophilic mulberry fibres, which are partially exposed at the surface. After coating, the contact angle increased to 94° and has a lower decrease of 3 % after 2 min. The increased contact angle and the low percentage of decrease over time show the successful coating of the reinforced CG sheet. Furthermore, the achieved contact angle exceeds that of bovine leather, which has a contact angle of 92° after 5 s and a decrease of 14 % after 2 min.

Table 7: Measured water contact angle after an exposure time of 5, 30,60 and 120 s and the calculated decrease for the produced GC sheets and references.

	$\boldsymbol{\theta}$ [°]				
Sample	**5 s**	**30 s**	**60 s**	**120 s**	**Decrease [%]**
CG with glycerol	85 ± 4	82 ± 4	81 ± 4	79 ± 4	3
MB-40%-4min without coating	71 ± 6	64 ± 6	62 ± 5	58 ± 5	19
MB-40%-4min with coating	94 ± 3	93 ± 3	93 ± 3	91 ± 3	3
PU leather	118 ± 7	110 ± 8	106 ± 9	99 ± 10	16
Bovine leather	92 ± 5	86 ± 6	83 ± 6	80 ± 6	14

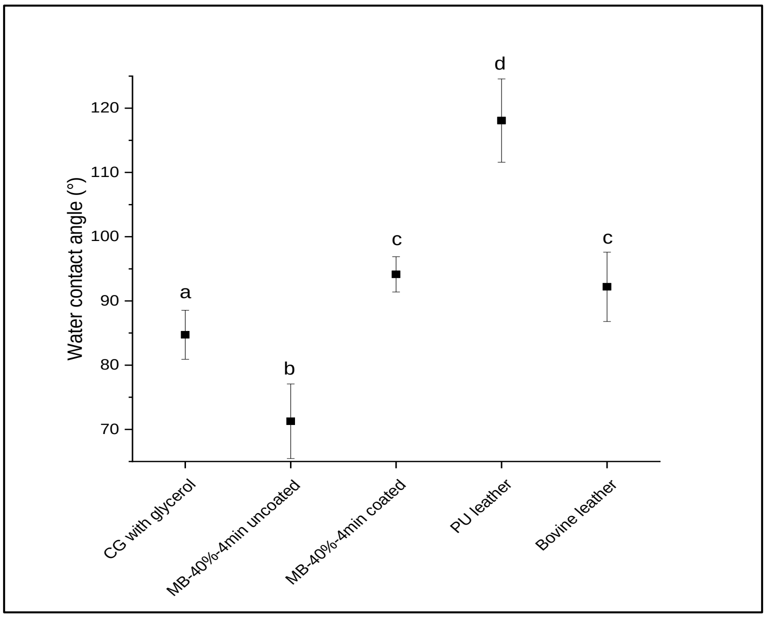


Figure 10: Comparison of the mean water contact angles of the produced CG sheets with and without reinforcement with mulberry fibre and the PU and bovine leather after an exposure time of 5 s. The ANOVA test and Tukey post-hoc analysis showed significant differentiation between all mean values, except when comparing the coated MB-40%-4min and bovine leather (p = 0.86). Mean values with the same letter did not show significant deviations.

The artificial leather made of PU achieved the highest contact angle at 118° after 5 s, which decreased to 99°. This high contact angle could be due to the rough morphology of the artificial leather, which mimics suede leather and provides a Cassie-Baxter wetting regime, i.e. air inclusions under droplets.[45] However, the contact angle decreased by 16 %, which is higher than the decrease of the coated MB-40%-4min. To determine significant differences in water contact angles after an exposure time of 5 s, ANOVA and Tukey post-hoc analysis were performed. As shown in Figure 10, the analysis concluded a significant difference between the contact angles among all sheets, except for the comparison between the coated MB-40%-4min and bovine leather (p = 0.86).

4.2. Cosmetic sheet masks derived from fungal biomass

For the cosmetic sheet masks, the three fungi species, namely white button mushroom, Lion's mane and Shiitake, were tested. Two different extraction methods, one with dist. H_2O and one with 0.1M NaOH were used. All of the prepared GC sheets were highly flexible and foldable and are shown in Figure 11. Foldability is particularly important since cosmetic masks have to be packaged in a folded format. The prepared CG masks extracted from white button mushrooms were light brown and felt soft and smooth on the skin. As shown in Figure 11, the surface of CG sheets extracted using 0.1M NaOH was even, while the surface of CG sheets extracted with H_2O showed more inhomogeneities. This could be because fewer extractives were removed from the CG complex during extraction with H_2O than with 0.1M NaOH. This could result in lower connectivity between the chitin-glucan nanofibers, leading to inhomogeneous film formation. CG sheets prepared from Lion's mane showed a bright yellow colour. The dark brown particles within the sheets originate from the substrate, which was improperly removed. The surfaces were soft but slightly rougher than the sheets made from white button mushrooms and Shiitake. The colour of CG sheets extracted from Shiitake is dark brown with black particles originating from the fruiting body. CG sheets from Shiitake were more rubbery than those from other fungi species and had a slight smell of Shiitake.

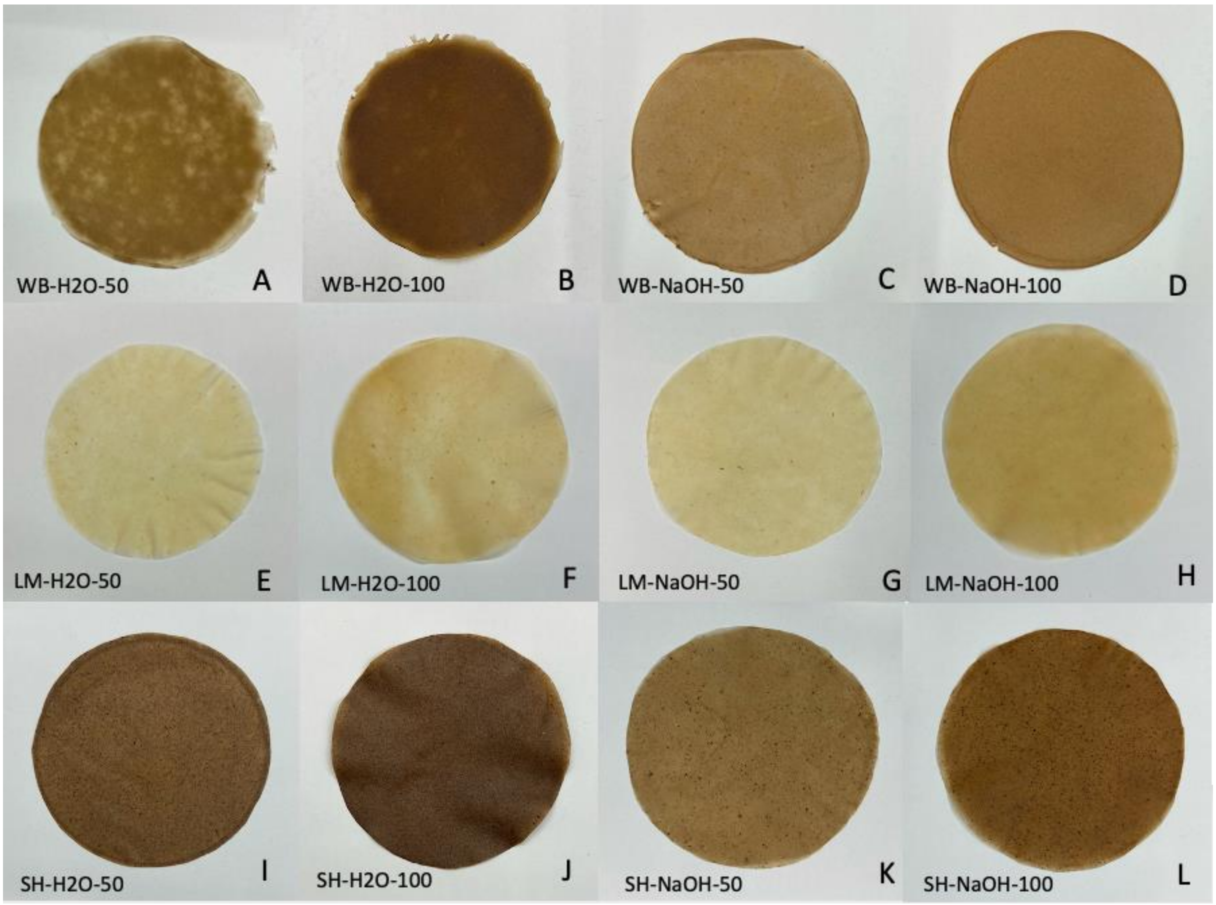


Figure 11: CG sheets prepared from extracts from white button mushrooms (A-D), Lion's mane mushrooms (E-H) and Shiitake mushrooms (I-L) using dist. water or 0.1M NaOH with grammages of 50 and 100 gsm.

4.2.1. Characterisation of CG sheets for fungi-based cosmetic masks

The prepared CG sheets and a commercial cosmetic mask were characterised by grammage (gsm) and thickness (d). The results are shown in Table 8 for each fungi species. The aimed grammages of 50 and 100 gsm were exceeded due to the addition of glycerol and unremoved substrate. CG sheets extracted with 0.1M NaOH showed a higher grammage than sheets extracted with H_2O except for WB-H_2O-100 and WB-NaOH-100. The commercial cosmetic mask is a low-cost, one-way mask made of a thinly woven cotton sheet, explaining its low grammage. Furthermore, the envelope and skeletal density and porosity were measured. A high porosity of facial masks is desired to achieve high liquid absorption, successful incorporation and fast release of active substances.[46] Table 8 shows that the extraction method has a great influence on the porosity of the CG sheets.

Table 8: Grammage (gsm), thickness (d), envelope density (ρ_e), skeletal density (ρ_s) and porosity (Φ of the reference and the produced sheets.

Sample	**gsm [g/m²]**	**d [μm]**	**ρ_e [g/cm³]**	**ρ_s [g/cm³]**	**Φ [%]**
White button mushroom					
WB-H_2O-50	75.0 ± 3.6	68.1 ± 2.8	1.10 ± 0.02	1.39 ± 0.004	20.6 ± 1.5
WB-NaOH-50	87.0 ± 1.2	96.7 ± 4.1	0.90 ± 0.04	1.30 ± 0.010	30.5 ± 2.8
WB-H_2O-100	194.9 ± 4.1	160.8 ± 4.7	1.21 ± 0.02	1.34 ± 0.001	9.7 ± 1.5
WB-NaOH-100	140.7 ± 5.6	141.3 ± 7.6	1.00 ± 0.03	1.15 ± 0.015	13.1 ± 2.7
Lions‘ mane					
LM-H_2O-50	74.7 ± 12.3	85.3 ± 11.7	0.88 ± 0.07	1.39 ± 0.002	37.2 ± 4.9
LM-NaOH-50	142.2 ± 2.9	116.3 ± 5.0	1.22 ± 0.03	1.35 ± 0.004	9.3 ± 2.2
LM-H_2O-100	122.2 ± 21.9	123.6 ± 30.6	1.01 ± 0.09	1.37 ± 0.002	26.7 ± 6.9
LM-NaOH-100	277.7 ± 9.6	212.5 ± 7.2	1.31 ± 0.04	1.33 ±0.002	1.9 ± 2.8
Shiitake					
SH-H_2O-50	69.4 ± 2.5	82.1 ± 4.9	0.85 ± 0.03	1.02 ± 0.011	17.3 ± 2.9
SH-NaOH-50	85.3 ± 10.0	81.1 ± 6.9	1.054 ± 0.10	1.41 ± 0.003	25.3 ± 6.9
SH-H_2O-100	154.2 ± 5.9	137.5 ± 6.0	1.12 ± 0.03	1.23 ± 0.008	8.9 ± 2.7
SH-NaOH-100	179.9 ± 12.3	151.5 ± 8.5	1.19 ± 0.04	1.35 ± 0.004	12.1 ± 2.8
Commercial cosmetic mask	45.6 ± 8.3	157.4 ± 15.0	0.29 ± 0.05	1.77 ± 0.008	83.6 ± 3.0

Among the CG sheets extracted with H_2O, LM-H_2O-50 and LM-H_2O-100 achieved the highest porosities of 37% and 27 %. On the contrary, the porosity of CG sheets from lion's mane is reduced when using an extraction method with 0.1M NaOH and decreased to 9 % and 2 %. However, in the case of white button mushrooms, the extraction with 0.1M NaOH led to higher porosities. WB-NaOH-50 and WB-NaOH-100 showed the highest porosity among the CG sheets extracted with 0.1M NaOH with values of 31 % and 13 %. The commercial cosmetic mask exceeded the porosity of all CG sheets, resulting from the thin woven material. In addition, the target grammage affects the porosity as well. In all cases, the porosity of CG sheets with a target grammage of 100 gsm showed lower porosities than those with a target grammage of 50 gsm. This might be due to the higher amount of glycerol that remains in thicker films during vacuum filtration.

4.2.2. Mechanical properties of CG sheets for fungi-based cosmetic masks

Using tensile tests, the influence of the grammage and extraction method on the mechanical properties was evaluated. The measured tensile strength, modulus and strain to failure of the produced sheets and the commercial cosmetic mask are shown in Table 9. It can be seen that the grammage and, thus, thickness significantly influence the tensile strength and Young's modulus. When the same extraction method was used, sheets with a target grammage of 50 gsm reached higher tensile strength than sheets with a target of 100 gsm. On the contrary, the strain to failure is increased with higher grammage. This aligns with the assumption that more glycerol is retained in sheets with higher grammage since glycerol increases the elasticity of the produced sheets. The commercial cosmetic mask reached the lowest tensile strength and modulus but a high tensile strain of 41.2 %. Furthermore, the extraction method affects the mechanical properties of each fungi species differently.

Table 9: Tensile strength (σ), modulus (E) and strain to failure (ε) of the produced sheets.

Sample	σ [MPa]	E [MPa]	ε [%]
White button mushroom			
WB-H_2O-50	12.3 ± 1.4	134.6 ± 25.2	20.6 ± 2.0
WB-NaOH-50	6.8 ± 0.7	186.5 ± 18.0	10.4 ± 1.3
WB-H_2O-100	6.8 ± 1.0	43.7 ± 9.4	25.3 ± 1.7
WB-NaOH-100	3.2 ± 0.4	53.0 ± 12.1	12.2 ± 0.7
Lion’s mane			
LM-H_2O-50	5.8 ± 1.1	191.5 ± 77.2	11.8 ± 2.6
LM-NaOH-50	6.4 ± 1.0	86.3 ± 32.6	35.3 ± 2.5
LM-H_2O-100	4.9 ± 0.3	93.5 ± 18.6	19.7 ± 0.6
LM-NaOH-100	4.0 ± 0.2	21.0 ± 3.4	44.9 ± 3.2
Shiitake			
SH-H_2O-50	7.7 ± 0.6	195.6 ± 30.0	15.0 ± 1.2
SH-NaOH-50	11.9 ± 3.2	489.2 ± 211.0	8.0 ± 3.8
SH-H_2O-100	6.6 ± 0.8	80.0 ± 13.4	23.2 ± 2.6
SH-NaOH-100	5.6 ± 0.8	46.8 ± 14.5	23.2 ± 2.0
Commercial cosmetic mask	3.6 ± 1.4	0.004 ± 0.003	41.2 ± 9.5

Figure 12 visualises the representative stress-strain curves of CG sheets from white button mushrooms. It can be observed that the extraction in dist. water resulted in a higher tensile strength and strain to failure for the same grammage. WB-H_2O-50 and WB-H_2O-100 reached a tensile strength of 12.3 and 6.8 MPa and a strain to failure of 20.6 and 25.3 %. The extraction in 0.1M NaOH resulted in a higher modulus for WB-NaOH-50 with 186.5 MPa. For sheets from Lion’s mane mushroom, the extraction in 0.1M NaOH resulted in a higher tensile strain for the same grammage, as shown in Figure 13. LM-NaOH-50 and LM-NaOH-100 showed a max. tensile strain of 35.3 % and 44.9 %, exceeding the value of the commercial cosmetic mask with a strain to failure of 41.2 %. LM-NaOH-50 also showed the highest tensile strength of 6.4 MPa. Furthermore, sheets prepared form Lion’s mane and extracted with dist. water were stiffer and possessed higher moduli than sheets extracted with NaOH.

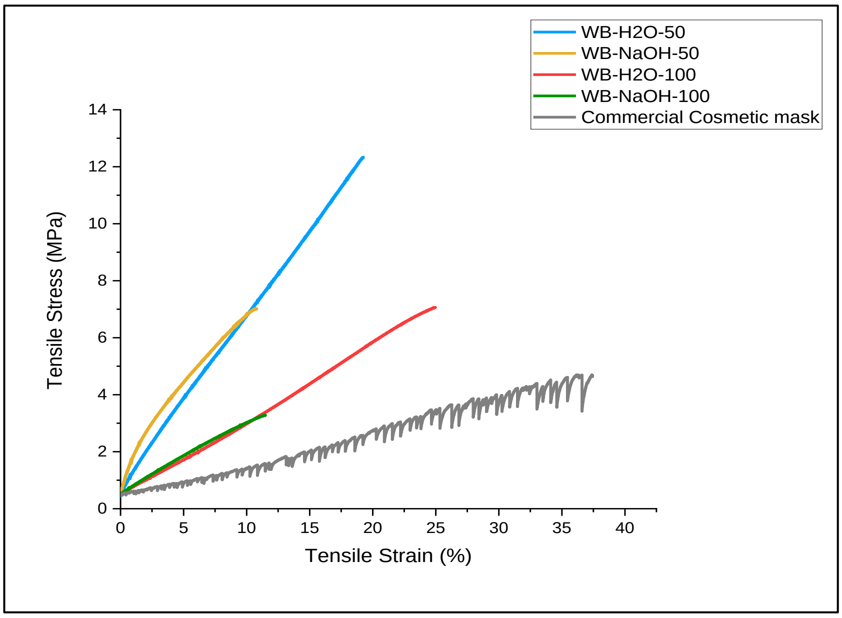


Figure 12: Representative stress-strain curves of produced CG sheets prepared from extracts from white button mushrooms using dist. water and 0.1M NaOH with grammages of 50 and 100 gsm and a commercial cosmetic mask.

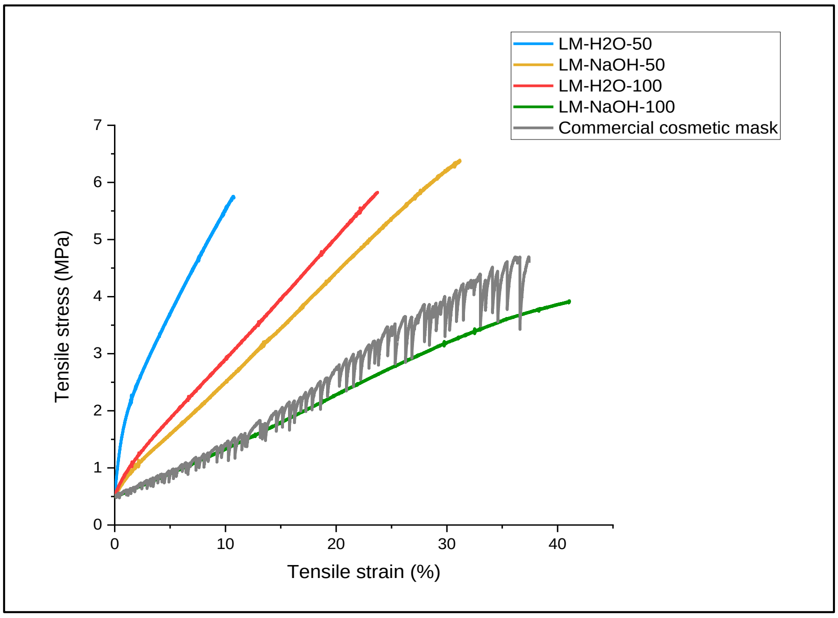


Figure 13: Representative stress-strain curves of produced CG sheets prepared from extracts from Lion's mane mushroom using dist. water and 0.1M NaOH with grammages of 50 and 100 gsm and a commercial cosmetic mask.

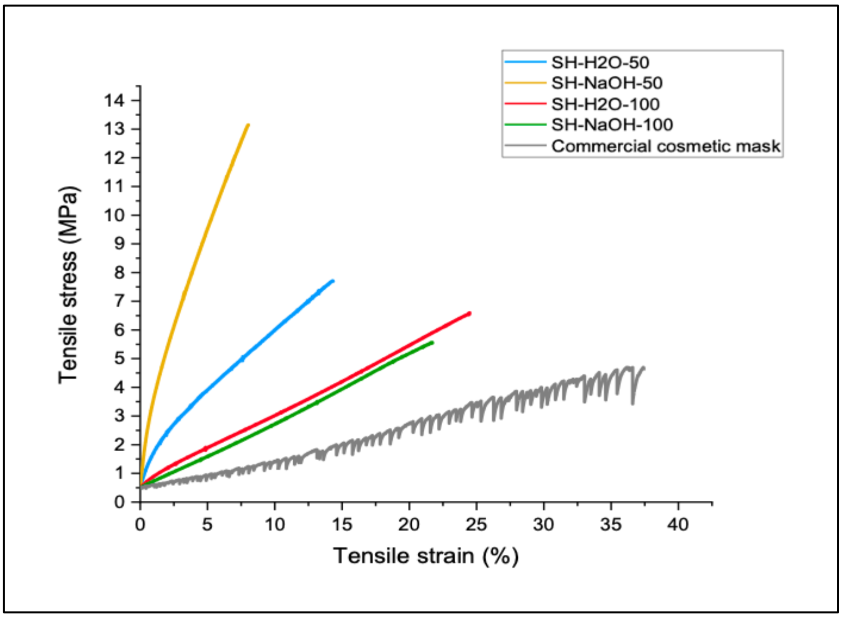


Figure 14: Representative stress-strain curves of produced CG sheets prepared from extracts from Shiitake using dist. water or 0.1M NaOH with grammages of 50 and 100 gsm and a commercial cosmetic mask.

In the case of sheets from Shiitake mushrooms (Figure 14), the extraction with dist. water resulted in higher strain to failure with 15.0 % for SH-H_2O-50 and 23.2 % for SH-H_2O-100 when comparing the same grammage. When comparing the sheets with a target grammage of 50 gsm, the sheet extracted with NaOH resulted in a higher tensile strength and reached the highest modulus among all sheets with 489.2 MPa. For sheets with 100 gsm, the extraction with H_2O resulted in a higher tensile strength and modulus.

All produced CG sheets exceeded the tensile strength and modulus of the commercial cosmetic mask, except for WB-NaOH-100. The sheets LM-NaOH-50 and LM-NaOH-100 showed tensile strains comparable to the commercial mask. Thus, sheets prepared from lion’s mane mushrooms using NaOH for extraction are potentially suitable candidates for application as cosmetic masks in terms of mechanical properties.

4.2.3. Water uptake of CG sheets for fungi-based cosmetic masks

One of the main purpose of a cosmetic sheet mask is to hydrate the skin. As a dry sheet mask, the CG sheets need to be soaked in water or serum prior application. For this reason, the water uptake was measured directly after soaking in water and after drying at room temperature. It was assumed that the consumer need to soak the dry sheet in water for 3 min and then apply the sheet mask for 10-20 min. The water uptake after every 5 min and the decrease after 20 min are summarised in Table 10. The results show high standard deviations due to the manual execution of this experiment and the fluctuating remaining water on the sample after wiping off the excess water. Therefore, these results should initially give a first insight into the water uptake of the samples.

Table 10: Water uptake of the reference and the produced sheets right after soaking in water for 3 minutes and after a drying time of 5, 10, 15 and 20 min.

Sample	**Water uptake after soaking in water [wt.%]**	**Water uptake after 5 min [wt.%]**	**Water uptake after 10 min [wt.%]**	**Water uptake after 15 min [wt.%]**	**Water uptake after 20 min [wt.%]**	**Decrease after 20 min drying [%]**
White button mushroom						
WB-H_2O-50	232.0 ± 34.4	195.6 ± 34.4	149.5 ± 36.8	102.6 ± 39.2	64.9 ± 39.6	72.0
WB-NaOH-50	308.1 ± 7.2	272.7 ± 8.1	234.2 ± 7.7	191.6 ± 9.3	149.2 ± 11.2	51.6
WB-H_2O-100	130.7 ± 13.9	112.6 ± 11.5	96.0 ± 9.8	77.1 ± 10.1	60.3 ± 9.9	53.9
WB-NaOH-100	137.3 ± 11.1	123.3 ± 12.3	100.3 ± 9.6	82.5 ± 9.3	64.6 ± 9.3	52.9
Lion's mane						
LM-H_2O-50	270.2 ± 76.4	193.0 ± 86.1	127.2 ± 90.4	66.9 ± 88.0	30.3 ± 76.1	88.8
LM-NaOH-50	229.1 ± 43.8	202.0 ± 44.1	170.6 ± 43.7	138.5 ± 42.9	112.1 ± 41.0	51.1
LM-H_2O-100	89.2 ± 0.72	58.4 ± 3.7	23.2 ± 8.0	7.6 ± 0.16	3.33 ± 1.9	96.3
LM-NaOH-100	122.6 ± 7.5	106.6 ± 7.2	89.3 ± 6.4	71.3 ± 5.6	55.2 ± 5.2	55.0
Shiitake						
SH-H_2O-50	266.0 ± 25.3	212.9 ± 25.2	161.7 ± 25.2	108.7 ± 26.4	54.4 ± 24.3	79.5
SH-NaOH-50	217.5 ± 37.5	168.7 ± 31.4	108.5 ± 29.2	57.4 ± 23.2	21.2 ± 17.7	90.3
SH-H_2O-100	150.6 ± 11.6	125.4 ± 10.6	97.0 ± 11.6	72.6 ± 12.0	54.3 ± 10.6	63.9
SH-NaOH-100	136.5 ± 23.7	112.2 ± 24.0	87.9 ± 22.3	63.6 ± 22.6	43.3 ± 21.8	68.3
Commercial cosmetic mask	1431.0 ± 543.7	1369.7 ± 542.2	1303.8 ± 542.3	1223.9 ± 543.0	1121.8 ± 633.1	21.6

By comparing the water uptake right after soaking in water, it can be seen that sheets with a target grammage of 50 gsm showed higher water uptake than sheets with a grammage of over 100 gsm. This could be explained by the higher porosity of sheets for example for WB-H_2O-50 with a porosity of 21 % compared to WB-H_2O-100 with a porosity of 10 %. A higher number of pores allows the sheets to absorb more water. The lowest decrease in water retention among the produced sheets was achieved by WB-NaOH-50 and LM-NaOH-50 %, with 51.6 and 51.1 %. However, the water uptake of the produced sheets was not comparable to the commercial sheet mask, with 1431.0 wt.% water uptake after soaking in water and a decrease of 21.6 % after 20 min of drying. This can be explained by the high porosity of the commercial mask (see Chapter 4.2.1) and the more hydrophobic nature of chitin.

Figure 15 shows the resulting water uptake right after soaking in water for sheets with a target grammage of 50 gsm. The extraction method using H_2O resulted in significantly higher water uptake than the extraction with 0.1M NaOH in the case of sheets from Lion's mane ($p < 0.05$) and Shiitake mushrooms ($p < 0.05$). On the contrary, no significant difference was found between WB-H_2O-50 and WB-NaOH-50 ($p = 0.083$).

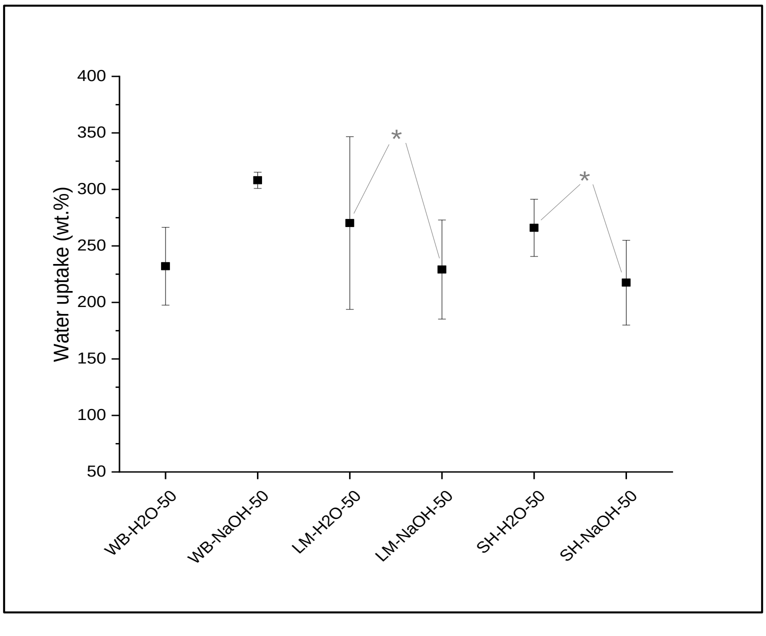


Figure 15: Water uptake of the produced sheets with a target grammage of 50 gsm right after soaking in water for 3 min. Significant differences were concluded between LM-H_2O-50 & LM-NaOH-50 ($p < 0.05$), SH-H_2O-50 & SH-NaOH-50 ($p < 0.05$), but not between WB-H_2O-50 & WB-NaOH-50 ($p = 0.083$).

The resulting water uptake right after soaking in water for sheets with a target grammage of 100 gsm is shown in Figure 16. Unlike sheets from Shiitake, where the extraction in H_2O resulted again in significantly higher water uptake ($p < 0.05$), sheets from Lion's mane mushroom showed higher water uptake when extracted with 0.1M NaOH ($p < 0.05$). Again, the sheets derived from white button mushrooms did not differ significantly between the extraction methods ($p = 0.630$). The comparison of the water uptake between the three different fungi types is illustrated in Figure 17 and Figure 18. As shown in Figure 17 the water uptake of CG sheets extracted with dist. water was not significantly influenced by the used fungi type. However, the fungi type played a greater role in the water uptake when the extraction was performed with 0.1M NaOH (Figure 18). In this case, the highest water uptake over time was achieved by sheets extracted from white button mushrooms.

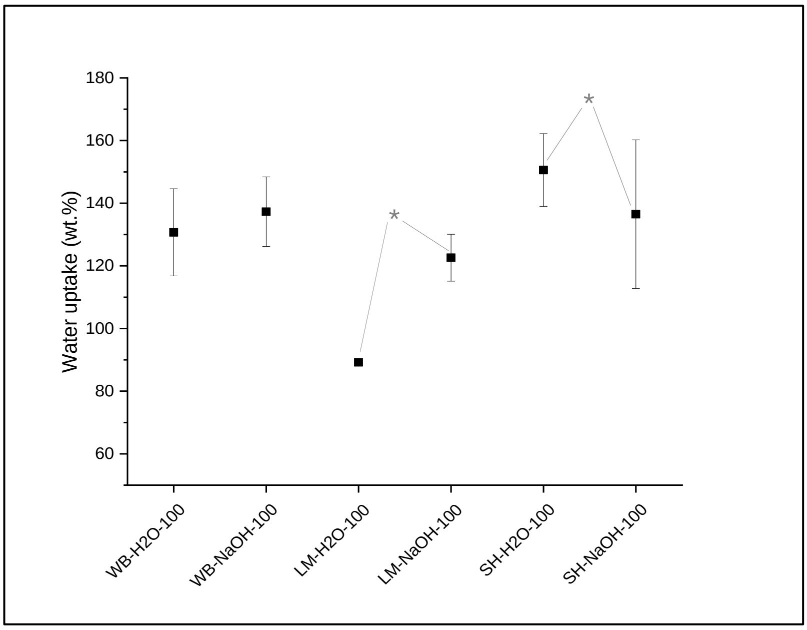


Figure 16: Water uptake of the produced sheets with an aimed grammage of 100 gsm right after soaking in water for 3 min. Significant differences were concluded between LM-H_2O-100 & LM-NaOH-100 ($p < 0.05$), SH-H_2O-100 & SH-NaOH-100 ($p < 0.05$), but not between WB-H_2O-50 & WB-NaOH-50 ($p = 0.630$).

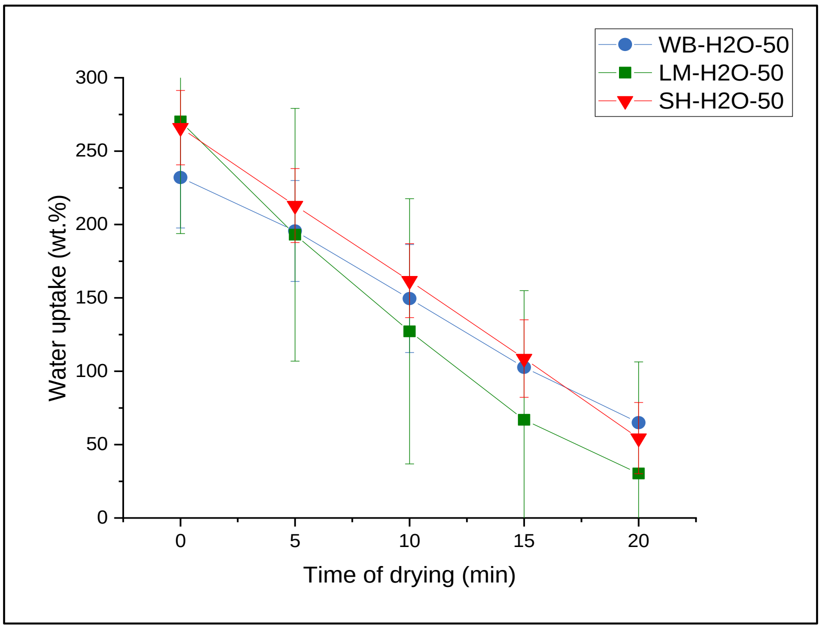


Figure 17: Water uptake of CG sheets prepared from extracts from white button mushrooms, Lion's mane and Shiitake with 50 gsm extracted using dist. water directly after soaking in water and after a drying time of 5, 10, 15 and 20 min.

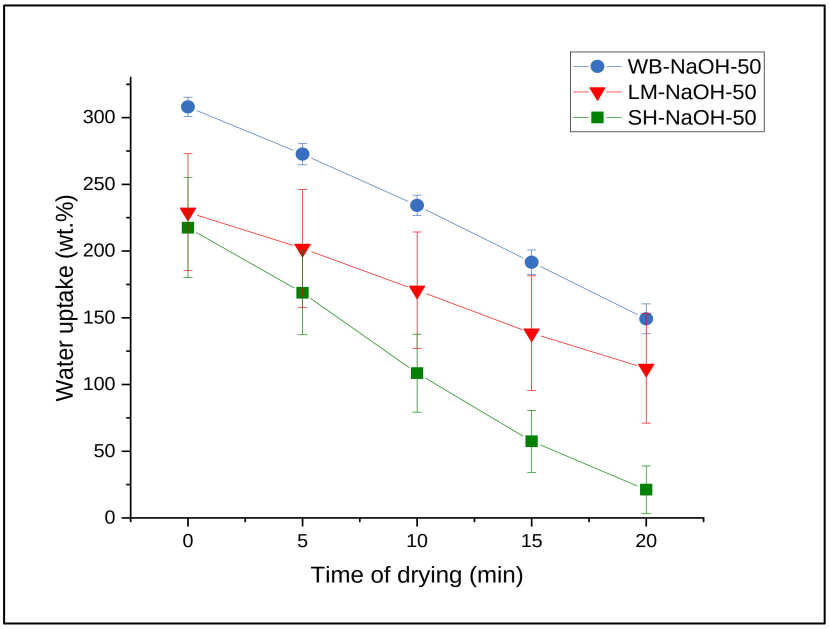


Figure 18: Water uptake of CG sheets prepared from extracts from white button mushrooms, Lion's mane and Shiitake with 50 gsm extracted using 0.1M NaOH directly after soaking in water and after a drying time of 5, 10, 15 and 20 min.

5. Conclusions

This project aimed to produce and characterise materials derived from fungal biomass for use as leather substitutes and cosmetic face masks. For this, the preparation pathway of chitin-glucan sheets was optimised to improve material properties regarding the intended applications. To prepare leather-like materials, the CG complex of white button mushrooms was extracted using 0.1M NaOH to remove water- and alkali-soluble components. To develop a flexible yet strong material, glycerol and fibres for reinforcement were added during CG sheet preparation. The reinforcement of CG sheets with mulberry fibres resulted in flexible and smooth sheets with homogeneously distributed fibres. This was not true for lyocell fibres. It was shown that higher porosities could be achieved with increasing weight fraction of mulberry fibres. Using tensile tests, I confirmed that the reinforcing fibres indeed improved the mechanical properties of CG sheets in terms of tensile strength and modulus. However, with increasing fibre content, the strain to failure decreased slightly. Furthermore, it was shown that different blending times slightly influenced the fibre length and sheet properties. The results showed that the fibre length was significantly lower after a blending time of 8 min. However, the results of tensile tests indicated that short blending times of 1 or 2 min do result in improved tensile strength compared to sheets prepared with longer blending times. The highest tensile strength of 19.6 MPa was achieved by a CG sheet reinforced with 40 wt.% mulberry fibres and a blending time of 2 min. These values are comparable with bovine and PU leather. The highest tensile strain of 15.3 % was reached by the CG sheet reinforced with 10 wt.% mulberry fibre and a blending time of 4 min. These findings indicate that chitin-glucan sheets derived from fungi hold great potential in producing materials resembling leather. It was possible to enhance sheet properties by improving the tensile strength with suitable reinforcing fibres and increasing hydrophobicity using coating materials.

For the production of fungi-based sheet masks, the three fungi types, namely white button mushroom, Lion’s mane and Shiitake, were investigated. CG sheets of two different grammages (50 & 100 gsm) were prepared by extracting the CG complex with 0.1M NaOH or dist. water. Both extraction methods resulted for all fungi types in flexible, smooth and foldable sheets. The extraction method, target grammage and fungi type, greatly impacted the porosity, moisture content and mechanical properties of the prepared sheets. Sheets with a lower grammage (target of 50 gsm) showed higher porosity and higher moisture content than sheets with a target grammage of 100 gsm. Furthermore, the tensile strength and modulus were higher for sheets with lower grammage. On the contrary, the strain to failure increased with higher grammage, which may be attributed to a greater amount of glycerol retained. The sheet properties of each fungi type were affected differently by the extraction method used. This suggests that the ideal extraction method depends on the used fungi type.

The highest tensile strength of 12.3 MPa was achieved by the 50 gsm CG sheet extracted from white button mushrooms using dist. water (WB-H2O-50). The highest strain to failure of 44.9 % was obtained for a 100 gsm CG sheet made using extracts from Lion's mane mushrooms using 0.1M NaOH (LM-NaOH-100), exceeding the values of the tested commercial cosmetic mask.

The results demonstrated that it is possible to produce eco-friendly cosmetic masks based on fungal biomass with comparable mechanical properties to commercial cosmetic masks. It was shown that sheet properties can be adjusted by using different extraction methods, grammages and fungi types.

This project highlights that chitin-glucan sheets derived from fungal fruiting bodies represent a promising sustainable alternative to synthetic materials and animal leather. The developed process shows the feasibility of producing low-cost, biodegradable and ethical-responsible materials with sheet properties, potentially suitable for applications in the cosmetic and fashion industry.

6. Future Work

The developed leather-like material derived from fungi showed comparable tensile strength to conventional bovine or artificial leather. However, in future steps, more improvement needs to be done to increase the strain to failure. In addition, a deeper investigation should be taken into the ventilating properties of leather. An example would be the determination of permeability to water vapour. A high permeability enables water removal from the inner atmosphere to the outside and prevents high temperatures and uncomfortable humidity during wearing.[39] Furthermore, the impact of moisture content on the mechanical properties should be determined in future steps.

For further investigation of fungi-derived cosmetic masks, an analysis of cosmetical active compounds after the extraction with dist. water and 0.1M NaOH should be performed. In addition, further research needs to be done to increase the moisture content after soaking in liquid. One way could be to increase the porosity of the masks or consider an application as a wet mask, where a longer soaking time in liquid could lead to higher moisture content during application. Finally, the physical attributes and sensation of a sheet mask are essential factors to consider. Therefore, sensory tests with volunteers, including adhesion and hydration effects on the skin[46], should be performed for the sheet masks to determine their suitability as facial cosmetic masks.

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Abstract

The textile industry, but especially fast fashion, is significantly impacting the environment, mainly due to using unsustainable sources for textiles, polluting production processes and the increasing amount of non-biodegradable textile waste. The urgent need to reduce the environmental footprint of the textile industry and the growing environmental awareness of society are leading to high demand for sustainable and biodegradable fabrics. Fungi-based materials have recently attracted great interest due to their environmental compatibility, low-cost cultivation, and unique mechanical properties.

This study investigated the development and characterisation of fungi-derived materials and their potential as alternatives to conventional leather and cosmetic sheet masks. Chitin-glucan, the structural co-polymer found in fungal cell walls, was mildly extracted using sodium hydroxide to produce leather-like materials. The chitin-glucan sheets were then prepared by resuspension and simple vacuum filtration. In addition, the material was reinforced with mulberry pulp fibres to improve mechanical properties. For the development of fungi-based cosmetic sheet masks, the chitin-glucan complex of three different fungi species was extracted. For this, two extraction methods, one with sodium hydroxide and one with dist. water, were investigated. The produced sheets were characterised by density, porosity and mechanical properties. Furthermore, the surface properties of the leather-like materials and the water-holding capacity of the fungi-based cosmetic sheet masks were analysed. The resulting fungi-derived leather-like materials showed promising mechanical properties and surface characteristics, with tensile strength and contact angles reaching or exceeding conventional leather materials. The fungi-derived cosmetic sheet masks showed tensile strength up to 12.3 MPa and strain to failure up to 44.9 %, comparable to commercial cosmetic masks. This study demonstrates the promising capabilities of fungi-derived chitin-glucan sheets for use as leather substitutes and cosmetic sheet masks. Furthermore, it emphasises that fungi-derived materials represent an eco-friendly and cost-effective alternative to conventional textile materials in the fashion and cosmetic sectors.

Zusammenfassung

Die Textilindustrie gilt als einer der größten Klimasünder weltweit. Die erheblichen Umweltauswirkungen entstehen dabei vor allem durch die Nutzung von Textilien aus nicht-erneuerbaren Ressourcen, der umweltbelastenden Produktionsprozesse und der zunehmenden Menge an biologisch nicht abbaubaren Textilabfällen. Die dringende Notwendigkeit, die Umweltbelastung der Textilindustrie zu reduzieren, zusammen mit dem wachsenden Umweltbewusstsein der Gesellschaft führt zu einer hohen Nachfrage an nachhaltigen und biologisch abbaubaren Textilien. Aktuell sind vor allem Materialien auf Basis von Pilzen aufgrund ihrer Umweltfreundlichkeit, dem kostengünstigen Anbau and der einzigartigen mechanischen Eigenschaften im Fokus der Wissenschaft.

Diese Studie untersuchte die Entwicklung und Charakterisierung von Materialien auf Basis von Pilzen und ihr Potenzial als Alternative zu herkömmlichen Leder- und Kosmetiktuchmasken. Für die Herstellung lederähnlichen Materialien, wurde das Chitin-Glucan, das strukturelle Co-Polymer in Pilzen, mit Natriumhydroxid extrahiert und anschließend durch Resuspension und Vakuumfiltration zu einem Film verarbeitet. Zudem wurde das Material mit Maulbeerfasern verstärkt, um die mechanischen Eigenschaften zu verbessern. Für die Herstellung der Kosmetiktuchmasken auf Pilzbasis wurde der Chitin-Glucan-Komplex aus drei verschiedenen Pilzarten extrahiert. Dabei wurden zwei Extraktionsmethoden, eine mit Natriumhydroxid und eine mit destilliertem Wasser, getestet. Die hergestellten Chitin-Glucan-Filme wurden anhand ihrer Dichte, Porosität und mechanischen Eigenschaften charakterisiert. Zusätzlich wurden die Oberflächeneigenschaften der lederähnlichen Materialien und das Wasserhaltevermögen der pilzbasierten kosmetischen Tuchmasken analysiert. Die pilzbasierten lederähnlichen Materialien wiesen vielversprechende mechanische Eigenschaften und Oberflächeneigenschaften auf, wobei die gemessene Zugfestigkeit und der Kontaktwinkel die von herkömmliche Ledermaterialien erreichten oder übertrafen. Die kosmetischen Tuchmasken auf Pilzbasis wiesen eine Zugfestigkeit von bis zu 12.3 MPa und eine Bruchdehnung von bis zu 44.9 % auf und sind somit vergleichbar mit kommerziellen kosmetischen Masken. Dieses Projekt zeigt die vielversprechende Verwendung von Pilz-basierten Materialien als Ledersatz und kosmetische Tuchmasken und unterstreicht deren Potenzial als umweltfreundliche und kosteneffiziente Alternative zu herkömmlichen Textilmaterialien in der Mode- und Kosmetikbranche darstellen.