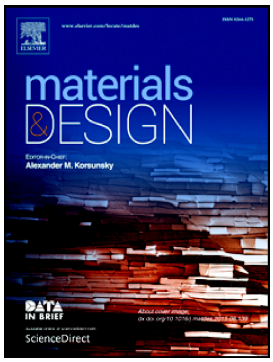

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Comparison of novel fungal mycelia strains and sustainable growth substrates to produce humidity-resistant biocomposites

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Abstract

Fungal mycelia are versatile, highly productive and sustainable sources for biocomposites to replace conventional plastics. However, with only very few fungal strains that have been characterized, numerous strains still remain unexplored as potential competitors against traditional non-biodegradable materials. Moreover, the functionality of mycelium composites at commonly occurring, challenging ambient conditions such as changing humidity and temperature is not well characterized. Here we evaluated the properties of the fungal composite material produced by novel fungal strains, including *Trichoderma asperellum* and *Agaricus bisporus*, grown on oat husk and rapeseed cake after oil pressing. The results showed that the mycelium composites were hydrophobic and strong, particularly when grown on rapeseed cake. *A. bisporus* grown on rapeseed cake exhibited increased stiffness after humidity was successively increased and decreased. The moisture-resistance of these novel mycelium composites is encouraging for novel sustainable material solutions.

Keywords: Mycelium biocomposites, fungal mycelia, rapeseed cake, dynamic mechanical analysis

1. Introduction

Use of numerous types of materials in commodities such as plastics and polymers is an essential aspect of modern living. These materials are expected to be resistant under varying conditions during transportation, storage, and product usage. Thus, simultaneous dynamic stresses such as load, temperature, and moisture affect all materials throughout their life cycle. Plastics such as expanded polystyrenes appeal to consumers because of their lightness, moisture-resistance, ease of handling, and in some cases low thermal conductivity [1]. However, the production of plastics

consumes non-renewable resources and energy. Moreover, difficulties in the disposal of such non-biodegradable plastic and polymeric materials after their intended use is considered as one of the most urgent environmental concerns [2]. Therefore, attempts to decrease the use of plastics and the amount of solid waste and environmental damage caused by pollution have given rise to the development of bio-based and biodegradable materials. For example, numerous recent studies have focused on the development of biocomposites that are reinforced with natural fibers from plant sources [3].

A recent alternative was introduced by directly growing mycelia with applicable substrates [4]. Construction of mycelium composites takes the basic inspiration from nature in terms of material creation *via* engineering [5]. Specifically, mycelium composites are produced by the complex network of fungal mycelium growing on a substrate of natural and/or waste resources. Filamentous fungi colonize the substrate when the internal turgor pressure and the rigid cell walls enable mycelial hyphae to penetrate organic materials, and the secreted enzymes degrade polymers of the substrate into molecules that can be taken up to serve as nutrients [6]. Therefore, mycelia function as a natural glue [7]. These biomaterials have a heterogeneous structure with a network of hyphae embedded in the feeding substrate material, which provides mechanical support for the resulting mycelia composite [8]. Mycelium composites are economically beneficial, as they are produced with a low level of energy, water, and from low-cost raw materials or low-grade agricultural by-products. As mycelium composites are biodegradable, they can be composted; they can also be re-used, for example as animal supplies, organic fertilizers, soil conditioners, and substrates for seedlings [8-10].

The substrates used to grow mycelium composites may be retrieved from industrial or agricultural waste streams, such as cereal straws, wood sawdust or other fibers, such as flax and cotton [11], or corn stove particles [12]. These by-products enable fungal mycelial growth, as they have moderate amounts of carbohydrates, lipids, proteins, inorganic compounds, and water [13]. The physical properties of the materials may show differences between the mycelium grown areas and undigested feeding substrate materials [14, 15]. Besides, several factors have been proposed to affect the properties of mycelium composites, including different combinations of fungal strains and substrates, and various treatments applied during the production processes.

Ganoderma lucidum and *Pleurotus ostreatus* are the most commonly used fungal strains for mycelium composites [4, 16, 17]. Hyphal architecture, fungal cell wall composition, composite constituents, and growth kinetics in mycelium composites are determined by inherent and exogenous factors, and vary widely among different species [17]. Among the different types, lignocellulose degrading basidiomycetes – particularly *Pleurotus ostreatus*, *Ganoderma lucidum*,

Ganoderma oregonense, *Lentinula edodes*, *Agrocybe aegerita*, and *Coprinus comatus* – are able to colonize and grow rapidly on various materials containing cellulose, hemicelluloses, and lignin [18]. Among these, *P. ostreatus and G. lucidum* specifically have been tested in mycelium composites. With over 5.1 million fungal species currently known [19], new fungal species may have unexplored potential if characterized in composite materials.

Mycelia composites are considered to be among the most promising alternatives to synthetic disposable materials such as expanded polystyrenes [9]. Potential applications include impact resistance, as well as thermal and acoustic insulation [10, 17, 20, 21]. Materials are commonly exposed to changes in humidity and temperature, sometimes rapidly and dramatically. In this respect, the characterization of different plant-based materials such as carboxymethylcellulose-based hydrogel [22], starch nanocomposite films [23], and cellulose/glucuronoxylan nanocomposite films [24] has shown that plant fiber-based materials commonly soften remarkably at high humidity. However, only few studies have analyzed the moisture uptake of mycelium composites [6, 25], hence a comprehensive understanding of the mycelia properties at challenging ambient conditions such as changing humidity and temperature is lacking. The present work aimed to 1) compare the growth and physical properties of mycelium composites from novel fungal strains grown on previously unused substrates, namely oat husk and rapeseed cake, and 2) characterize the novel mycelium composites at changing temperature and humidity, to thoroughly assess their material properties and evaluate the potential for their use at challenging conditions.

2. Materials and Methods

2.1. Materials

Fungal cultures

Trichoderma asperellum (TA) was isolated from a forest in Southern Finland; commercial *Agaricus bisphorus* (button mushroom, AB) and *Lentinula edodes* (shiitake) were purchased from a local grocery store. *Pleurotus ostreatus* (oyster mushroom, PO, HAMBI FBCC0515), *Ganoderma lucidum* (Reishi mushroom, GL, HAMBI FBCC665), *Pleurotus ostreatus sajor caju* (oyster mushroom, HAMBI FBCC471), *Pleurotus ostreatus florida* (oyster mushroom, HAMBI FBCC469), *Kuehneromyces mutabilis* (sheathed woodtuft, HAMBI FBCC2164), and *Flammulina velutipes* (enoki mushroom, HAMBI FBCC583) were obtained from HAMBI Culture Collection (University of Helsinki, Faculty of Agriculture and Forestry, Department of Microbiology).

All of the isolated fungal cultures were identified with internal transcribed spacer polymerase chain reaction (ITS-PCR). Genomic DNA was extracted from homogenized mycelia with a Phire Plant Direct PCR kit (Thermo Scientific, USA) according to the manufacturer's instructions. The

mycelial mass was homogenized with a mortar in liquid nitrogen before DNA extraction. The ribosomal DNA ITS region was amplified with the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer pair [26]. The PCR reaction (total 20 µl) contained 0.5 µl DNA template, 0.5 µm ITS1 and ITS4 primers, 10 µl 2 x Phire Plant PCR buffer and 0.4 µl Phire Hot Start II DNA Polymerase (Thermo Scientific). Initial denaturation was performed at 98ºC for 5 min followed by 40 cycles of (1) denaturation at 98ºC for 5 s, (2) annealing at 55ºC for 5 s and (3) extension at 72ºC for 20 s. The final extension was at 72ºC for 1 min. The concentration of the amplified PCR products was checked on a 1% agarose gel, and subsequently sequenced (Macrogen Corp., The Netherlands). The BLAST analysis was done against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to retrieve the ITS sequences with the highest identity.

The mycelia were propagated and maintained on 2% (w/w) malt extract (LabM, UK) 2% (w/w) agar (Scharlau, Spain) plates. All cultures were stored on malt extract agar at 4±1°C. The inoculum for the colonization growth (7 days) was made to liquid malt extract (2% w/w).

The feeding substrates for mycelia growth were oat husk (Fazer Mill, Finland) milled to 1 mm diameter with a centrifugal mill (Retch GmbH ZM100, Germany) and rapeseed cake (Avena Nordic Grain, Finland). Other substrates, namely oat husk without milling, pine sawdust milled to 1 mm (Pesäpuru, Pölkky Oy, Finland), oat straw (Merran Talli, Finland) chipped using a wood shredder (Eliet Machines, Belgium), and birch sawdust (Merran Talli, Finland) were also tested during the initial pre-screening study.

2.2. Pre-screening test for mycelia selection

All materials were autoclaved (Steris Finn Aqua, Finland) at 120°C for 20 min before their use. Feeding substrates were moisturized with tap water. The final water to substrate ratio (w/w) was as follows: oat husk 1:1, oat and birch sawdust 1:2, oat straw 1:2, rapeseed cake 4:3. They were autoclaved twice before inoculation with a minimum of one day between the autoclave sessions to better inactivate the contaminants.

Moisturized autoclaved feeding substrates were autoclaved on glass petri dishes. The fungi were inoculated to the substrate, and the dishes were sealed with Parafilm (Bemis, USA). The cultures were grown at 21±1°C in the laboratory cabinet. After 14 days, the cultures were visually inspected for quantitative and qualitative growth properties.

2.3. Growing of mycelia

Four selected fungal strains (TA, AB, GL, and PO) were first grown in 100 ml liquid malt extract for one week at 21°C in a 250 ml glass Erlenmeyer flask, covered with a cellulose plug and

aluminum cap, and sealed with Parafilm. Fungal inoculums (1 cm^3) were mixed with the feeding substrates (rapeseed (RS) and oat husk (OH)) and grown on petri dishes at 21ºC. After two weeks, the samples were visually inspected, mixed, and transferred to 4-well plates (1 cm^3/well) (Thermo Scientific, Denmark) and left to grow for one more week. The mycelium composites produced were then dried at 40ºC for 48 hours to result a water content between 5.8 and 8%. The resulting eight mycelium composites were abbreviated according to the fungal strain and growth substrate, as TA-RS, TA-OH, AB-RS, AB-OH, GL-RS, GL-OH, PO-RS, and PO-OH.

2.4. Chemical Characterization

Chemical characterization of the four selected fungal strains and the feeding substrates (RS and OH) as well as the biocomposites produced was performed with a Fourier transform infrared (FT-IR) spectrophotometer (Perkin Elmer Spectrum, USA) with the attenuated total reflectance (ATR) accessory. All spectra were recorded in the range from 4000 to 800 cm^{-1} with 4 cm^{-1} resolution, accumulating 128 scans [6].

2.5. Physical characterization

The four selected strains grown on OH and RS substrates to firm mycelium composites were physically characterized by the following analyses.

2.5.1. Water Uptake

Water absorption and desorption of the mycelium composites and the plain (before inoculation) substrates were measured using a Dynamic Vapor Sorption (DVS Intrinsic, Surface Measurement Systems Ltd., UK) microbalance. Measurements were made at 25ºC, at relative humidity conditions from 5% to 90%, in a stepwise procedure (240 minutes at each step), followed by a similar stepwise decrease from 90% to 5% [27].

2.5.2. Density

Density measurements of the mycelium composites were made by using glass beads (0.2 mm diameter, 1.68 g/cm^3) according to a previously described method [24]. All samples were conditioned for three days at 50% relative humidity (RH) before the density measurements.

2.5.3. Compressive strength

Compressive strength of mycelium composites was measured with a Texture Analyzer TA-XT2i (Stable Microsystems, UK) 36 mm probe at 35% strain, 5 kg force, pre-test speed of 1.0 mm/s and test speed of 2 mm/s. Compressive strength was determined from the obtained force-time profile and stress-strain curve. Mycelium samples had a diameter of 12–14 mm, and thickness of 6 mm. All samples were conditioned for three days at 50% RH before the compressive strength measurements.

2.5.4. Effect of temperature and humidity on mycelium composites

The thermal and mechanical properties of mycelium composites were evaluated by a Dynamic Mechanical Analyser (DMA) (TA Instruments, UK) using a compression clamp in the dynamic oscillatory mode (DMA multi-frequency/strain experiments). Using the humidity accessory, DMA measurements started with chamber conditioning at 30% RH for 5 hours at 25ºC. RH was first increased in a stepwise manner by 0.50% every 2 min until it reached 90% RH, and then back to 0% RH with the same rate.

After initial isothermal conditioning (25ºC at 50% RH for 4 hours), temperature scans were carried out by applying a slow ramp (0.1 N, 0.5ºC/min) between 25–70ºC and then cooling down to 25ºC at the same rate. The DMA runs were made at 1 Hz and 5 Hz frequencies.

3. Results and Discussion

3.1. Prescreening Test

A prescreening test was done to compare the growth rate of nine fungal strains on five different feeding substrates, the latter of which were by-products from agriculture or forest industry. The biocomposites were evaluated by testing the integrity of the formed structures, both visually and manually. According to the pre-screening trials (Table 1), oat husk (OH, 1 mm) and rapeseed cake (RS) were selected as the feeding substrates to be used in the further measurements, because they produced composites with high integrity. Of the mycelia strains, TA, AB, PO, and GL were selected for detailed characterization, since these strains grew rapidly and formed intact, homogeneous and rigid mycelium composite structures. TA and AB are previously unexplored strains in mycelium composites, and they were compared with the known strains of PO and GL to examine the unknown potential for bio-based composites and reveal any differences compared to the known strains. The other tested strains demonstrated slow growth rate and/or relatively weak structures, and thus they were eliminated from the remaining part of the study. Photographs of the selected mycelium composites are shown in Figure 1.

3.2.Chemical Characterization

The FT-IR spectra were measured to characterize the chemical composition of the studied mycelia and to compare the chemical nature of different strains and substrates. Mycelium-based materials grown on selected feeding sources are expected to inherit the microstructure and properties of the feeding material [8].

Table 1. Growth of fungal strains on different feeding substrates after 14 days

	Mycelia								
Feeding substrates	*Pleurotus ostreatus* (PO)	*Pleurotus o. sajor caju*	*Pleurotus o. florida*	*Ganoderma lucidum* (GL)	*Kuehneromyces mutabilis*	*Flammulina velutipes*	*Trichoderma asperellum* (TA)	*Agaricus bisporus* (AB)	*Lentinula edodes*
OH	++	+++	+++ (fluffy)	+++ (flat)	++ (fluffy)	+++	+++ (fluffy)	+++ (fluffy)	++
RS	++ (flat)	++	++	+ (flat)	++	+++ (flat)	+++ (fluffy)	++ (fluffy)	+(flat)

OH: Oat husk, RS: Rapeseed cake

Fluffy or flat growth morphology is indicated in parenthesis, if applicable.

The growth rate is indicated with plus signs: + slow, ++ moderate, and +++ fast growth.

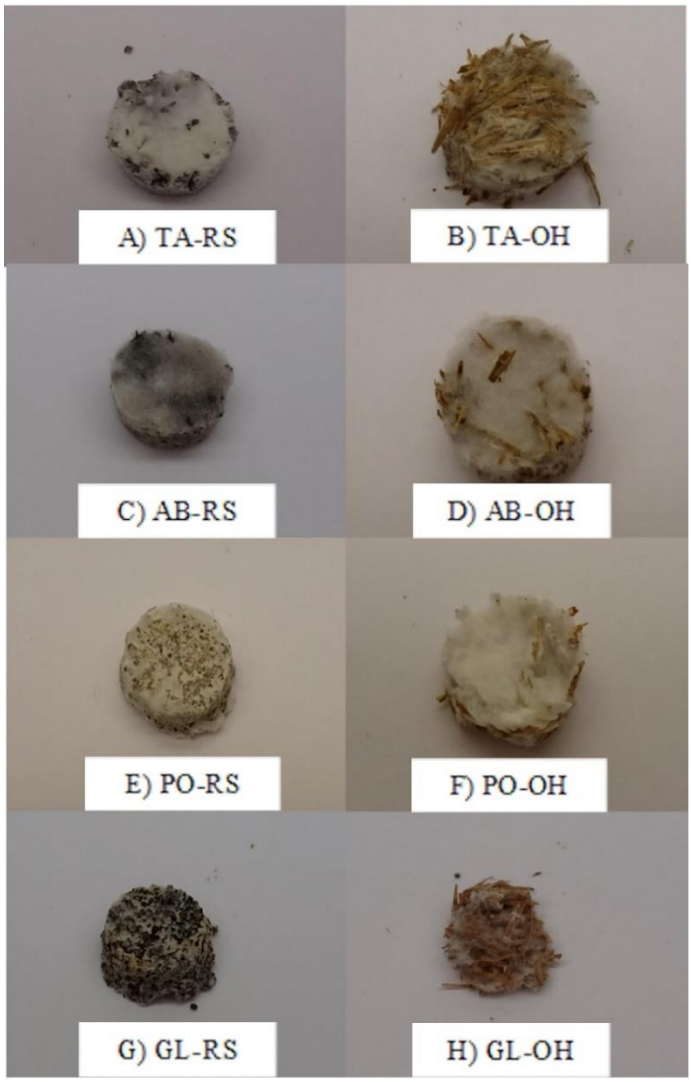


Figure 1. Images of the mycelium composites

Trichoderma asperellum (TA) grown on A) rapeseed cake (RS) and B) oat husks (OH) as substrates; *Agaricus bisporus* (AB) grown on C) RS and D) OH; *Pleurotus ostreatus* (PO) grown on E) RS and F) OH; *Ganoderma lucidum* (GL) grown on G) RS and H) OH.

Previously, the effect of different substrates on the polysaccharide, lipid, and chitin composition of mycelia films produced from *P. ostreatus* and *G. lucidum* has been reported [25].

The observed bands are presented in Table 2, and the spectra of the mycelia both as plain and as grown on substrates are shown in Supplementary Material (Figure S1). According to the FT-IR spectra, both the pre-inoculation mycelia and the plain RS and OH substrates had distinct differences in their chemical structures. Specifically, RS induced stronger peaks for polysaccharides, proteins, and lipids than OH.

The fungal cell wall is composed of various mannans, different types of β-glucans, chitin, and proteins. Some other polymers that possess carboxyl, phosphoryl, hydroxyl, amino, amine and

imidazole functional groups have also been detected on the surface [28, 29]. The characteristic functional groups in fungal mycelia are as follows: 3000–2800 cm^{-1} for fatty acids/lipids; 1700–1600 cm^{-1} for amide I, 1575–1300 cm^{-1} for amide II and amide III (proteins), and 1200–900 cm^{-1} for polysaccharides [30]. Differences in β-1,3-glucan structures are characterized by shoulders (1070 cm^{-1}) and/or slight deviations around 1090 cm^{-1} [30]. These are consistent with our findings. Before inoculation on substrates, the TA and GL mycelia gave generally the strongest absorption peaks among the studied mycelia strains (Supplementary Materials, Figure S1). In contrast, AB gave slightly greater intensities mainly for proteins and lipids when it was fed with OH as a substrate, in comparison to the plain mycelia before inoculation. GL showed a stronger indication of polysaccharides when fed with OH. The studied mycelia also differed in lipid structure when OH was used as the feeding substrate.

Table 2. Observed bands in the FT-IR (Fourier transform infrared) spectra. Adapted from [25]

Assignment	*Mycelium component (main contribution)*	**TA**	**AB**	**PO**	**GL**	**RS substrate**	**OH substrate**
O-H stretching	**Polysaccharides**	3289	3285	3278	3290	3284	3305
CH_2 asymmetric stretching	**Lipids**	2925	2924	2971	2930		
CH_2 symmetric/ asymmetric	**Lipids**		2854				
Ester C=O stretching	**Lipids**		1745				
Amide I (β-sheet)	**Proteins**	1639	1634	1649	1640	1631	
Amide II	**Proteins**			1547			
CH_2 bending	**Lipids**		1403			1443	
C-H bending	**Chitin**	1371	1370	1370	1377	1370	1370
Amide		1259		1313			
PO_2^- asymmetric stretching	**Nucleic acids**		1241	1230			
C-C stretching	**Polysaccharides**	1020	1025	1028	1020	1044	1020

TA: *Trichoderma asperellum*, AB: *Agaricus bisporus*, PO: *Pleurotus ostreatus*, and GL: *Ganoderma lucidum* mycelia, RS: rapeseed cake and OH: oat husk.

3.3.Density

Density is generally accepted as a good indicator of the mechanical properties of bio-based materials [31]. Mycelium composites are often considered as being similar to solid foams with their low density, high porosity, and slight rigidity [32]. The densities of mycelium-based foam-like materials may range from 59 to 318 kg/m^3 [17]. Extended polystyrene foams were considered as their primary competitor, with densities around 50 kg/m^3. On the other hand, higher densities of mycelium composites have been highlighted as a significant contributor to the elastic behavior of the materials, due to filling the micropores of the composite materials [9].

According to the present findings (Figure 2), the selection of growth substrate affected the density more than the mycelium strain. This is in agreement with a previous study [33] that compared densities of *Lentinus strigosus* (Schwein.) Fr. mycelia grown on wood sawdust samples of pitch (*Protium puncticulatum*), tauari (*Cariniana micrantha*), and piquiarana (*Caryocar glabum*) supplemented with wheat, corn, or rice bran [33]. In the present work, the RS-fed mycelium composites had higher densities than those that were OH-fed. This can be expected, as the density of RS has been reported to be around 561 kg/m^3 to 557 kg/m^3 [34] [35], while the density of OH was only about 230 kg/m^3 [36]. Moreover, the OH had a more porous structure in comparison to RS. Porosity is considered to be a significant factor that affects the densities of mycelium composites [20].

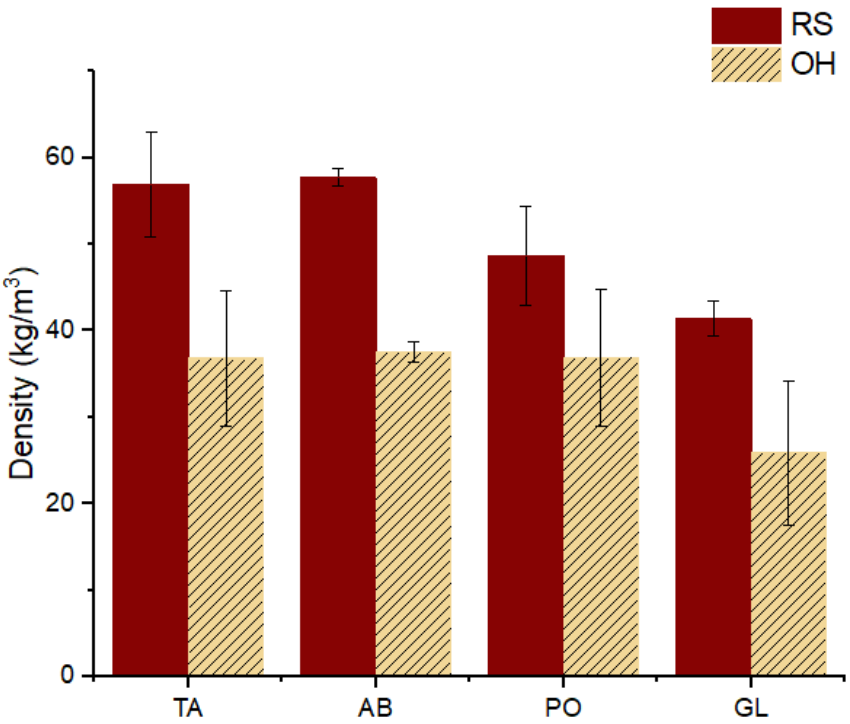


Figure 2. Density measurements of the mycelium composites

Values are means of duplicate measurements ± standard deviations. RS: rapeseed cake; OH: oat husk; TA: *Trichoderma asperellum*, AB: *Agaricus bisporus*, PO: *Pleurotus ostreatus* and GL: *Ganoderma lucidum* mycelia.

When grown on RS, the novel mycelium composites AB-RS and TA-RS showed higher densities than composites with the known strains, PO-RS and GL-RS. However, differences in densities measured among strains grown on RS and strains grown on OH were not statistically significant ($p>0.05$).

In previous studies, higher mycelial density has also been correlated with a higher amount of hyphae branching, which leads to a more compact structure with fewer micropores. More specifically, hyphae branching in GL – rather than lengthwise hyphae growth in PO – has been suggested as the cause for their density differences in film application [25]. This finding is in contrast with the present results, as the PO mycelia grown on both substrates showed slightly higher densities than GL. In mycelium composites, replacement of substrate with fungal material has been reported to decrease the density [37]. The availability of nutrients, such as carbon sources and/or supplementation [9], and differences in fungal growth rate also affect the material density; the extracellular enzymes released from fungal mycelia hyphae contribute to the growth by degrading the substrate used, and thus increase the mycelia density [9, 25].

3.4. Compressive strength

Compressive strength is one of the most significant material parameters, particularly for packaging applications that are intended to protect the inside contents from mechanical damage [17]. The studied mycelium composites differed in their compressive strengths (Figure 3). Specifically, the RS-fed mycelia composites showed a higher strength than those that were OH-fed. The novel TA-RS composite showed the highest compressive strength of all studied samples (299.6 kPa); this, along with the GL-RS and PO-RS (274.8 and 274.6, respectively) was significantly higher compared to AB-RS (200.2 kPa) ($p<0.05$). Stress-strain curves of mycelia composites are provided in Supplementary Materials (Figure S2). The present findings support the previous knowledge that the compressive strength of mycelium composites is highly dependent on the substrates and mycelia strains, which also affect the morphology of the mycelium composites [4]. Among the previously known strains, GL-RS showed higher compressive strength than PO-RS. The difference was more evident in the OH-fed composites of these strains. This is in agreement with a previous study, where *Ganoderma* demonstrated higher compressive strength than *P. ostreatus* spp. [38].

In the present study, the compressive strength ranged between 16.8 and 299.6 kPa. Therefore, mycelium composites may be considered as competitors of expanded polystyrene, as these have been reported to have the compressive strength in a similar range (69–400 kPa) [38, 39]. The

presence of chitin in the fungal cell wall has been suggested to provide mechanical strength to mycelium composites, as chitin is aggregated into fibrils that decrease the crack formation during compression and support the material structure [9, 40]. Proteins and lipids may function as plasticizers, while polysaccharides give stiffness to mycelium-based films [25]. In the present study, the chemical characterization by FT-IR highlighted the presence of polysaccharides in the RS-fed mycelium composites, which may contribute to the higher compressive strength of these composites.

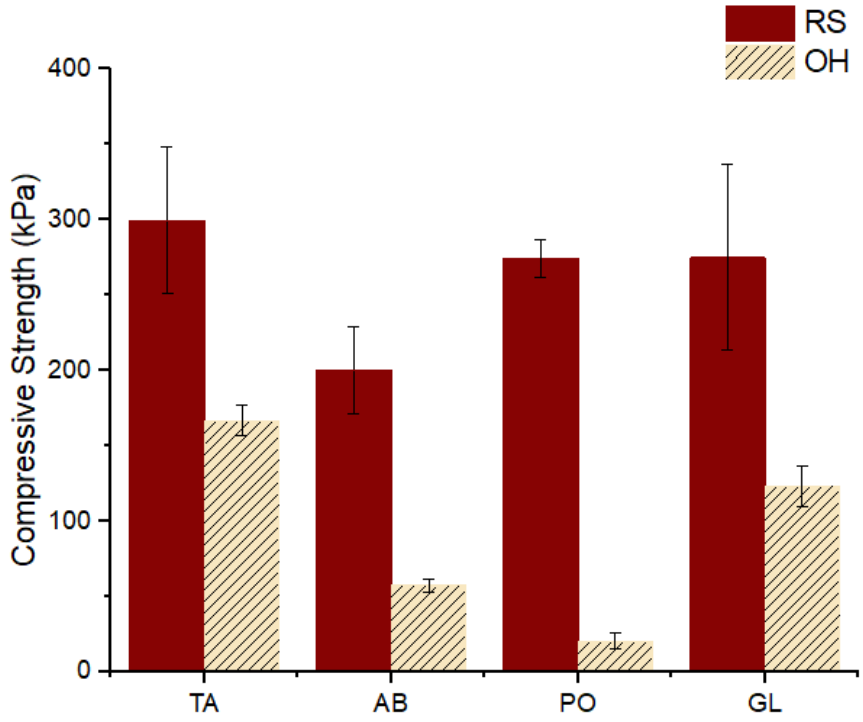


Figure 3. Compressive strength measurements of the mycelium composites

Values are means of duplicate measurements ± standard deviations. RS: rapeseed cake; OH: oat husk

TA: *Trichoderma asperellum*, AB: *Agaricus bisporus*, PO: *Pleurotus ostreatus*, and GL: *Ganoderma lucidum* mycelia.

Density of the mycelium composites was not clearly correlated with their compressive strength. TA-RS showed both the highest density and compressive strength. However, the other high-density sample (AB-RS) had a relatively low compressive strength. Previously, high-density mycelium composites produced by white-rot fungi also revealed higher compressive strength [40]. However, similar to our findings, densities of mycelium-based materials do not generally correlate with their compressive strength measurements, as clearly outlined in a recent review [32]. In the present work,

the density was mainly affected by the feeding substrate (RS or OH), but it appears that the compressive strength depends also on the growth behavior of mycelia. Recently, Appels et al. [43] revealed that location of dominant growth of hyphae significantly affects the mechanical strength of mycelium composites. Presently, the TA mycelium, which had high compressive strength, showed clearly decreased FT-IR bands for polysaccharides after growing on the substrates (Supplementary Material, Figure S1). Further studies are needed to fully understand the connection between mycelia composition, growth, and mechanical properties of mycelium composites.

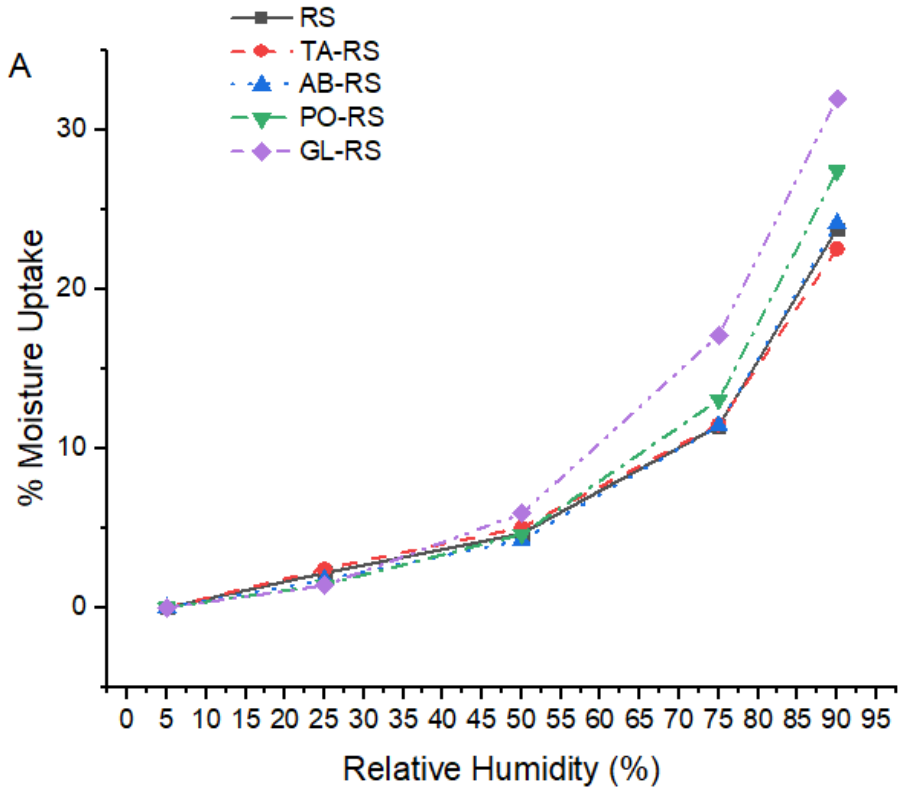
3.5.Water Uptake

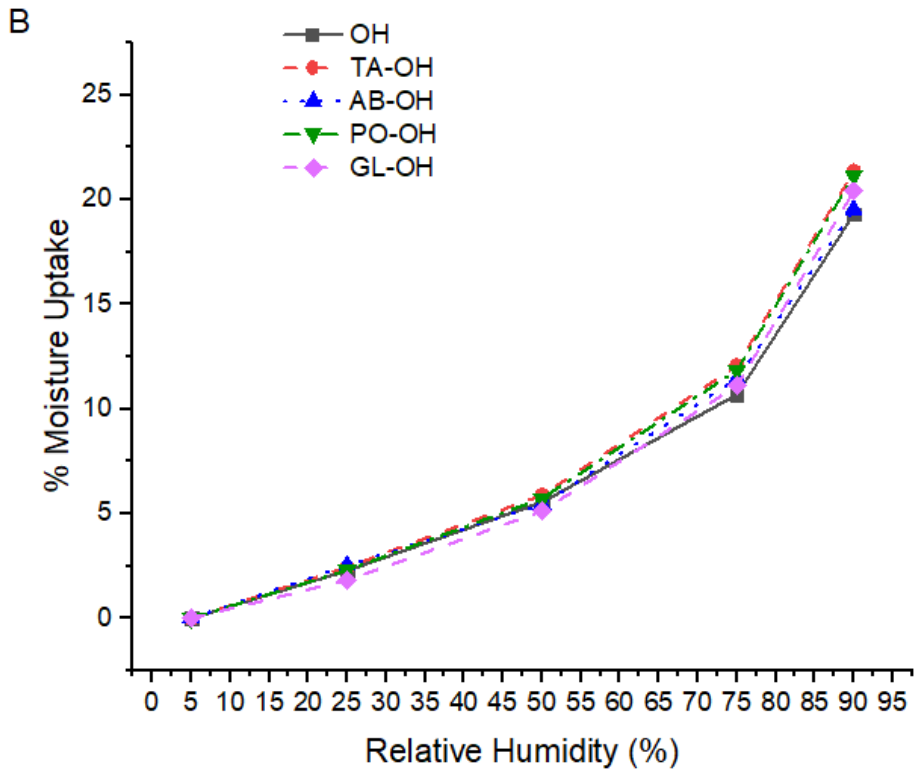
Water uptake is an inherent characteristic of materials, and influences their other properties, such as mechanical stiffness. Furthermore, water uptake may affect the use of materials as packaging applications, for example. Susceptibility to water might be more complex when considering composite materials compared to single-component systems [41]. In the present study, all mycelia composites, either with RS or OH as feeding substrates, had rather low (up to 5%) water uptake until 50% RH. Therefore, all samples – irrespective of the mycelium and substrate types – seemed to be resilient to humidity (Figures 4a to 4d). Substrate type did not determine the water uptake; rather, the mycelium matrix had a large effect on it. However, mycelium composites fed with RS had slightly higher water absorption than those fed with OH.

At 75% RH, the water uptake increased to about 10% for all composites except for GL-RS (17.1%). At the highest studied RH (90%), GL-RS showed the highest water uptake of 32.0%. Among the mycelium composites grown on OH, TA-OH had the highest water uptake of 21.4%. In contrast, AB-OH had the lowest water uptake at 90% RH (19.5%), and TA-OH had 22.6%.

Most fungi are considered as hydrophobic, a property that is linked to hydrophobins – low-weight proteins found only in fungi. Hydrophobins have several functions related to cell wall morphogenesis, hydrophobicity, and substrate adhesion [32]. The difference in water uptake mechanisms of different mycelia has been related to the difference in their chemical structure. The relatively low content of chitin in the *P. ostreatus* (PO) cell wall, with cellulose/potato-dextrose as a feeding substrate, has been attributed to its higher sensitivity to water uptake [25]. However, no clear relation was detected between the water absorption behavior of the present mycelium composites and their strength of chitin signals in FT-IR. An increase in water uptake at around 80% RH is commonly observed for most polysaccharides. The higher moisture uptake around this humidity level has previously been related to the onset of capillary condensation [42]. Mycelium composites of *P. ostreatus* and *T. multicolor* grown on rapeseed straw, exposed to 60% and 80%

RH conditions showed slightly lower weight gain results (3.87–8.22% at 60% RH and 10.00–10.96% at 80% RH [43]) than the present findings.





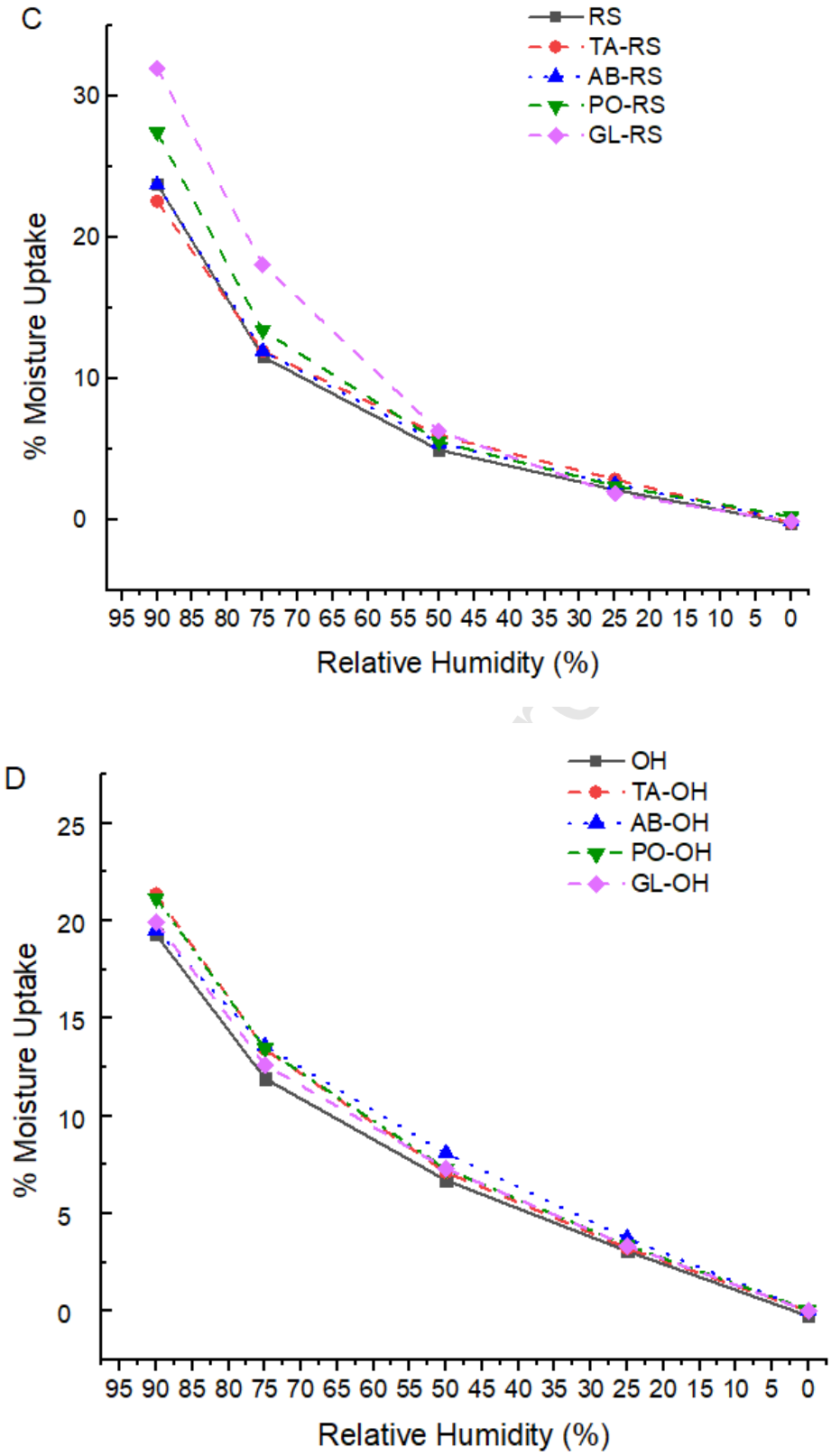


Figure 4. Moisture uptake in mycelium composites

A) mycelium composites grown on RS, with increasing relative humidity, B) mycelium composites grown on OH, with increasing relative humidity, C) mycelium composites grown on RS, with

decreasing relative humidity, and D) mycelium composites grown on OH, with decreasing relative humidity. TA: *Trichoderma asperellum,* AB: *Agaricus bisporus,* PO: *Pleurotus ostreatus,* and GL: *Ganoderma lucidum* mycelia.

Among the samples fed on RS, those that absorbed higher levels of water (GL-RS and PO-RS) maintained higher moisture during desorption until 50% RH. Below 50% RH, all samples showed a similarly gradual decrease.

3.6. Effect of temperature and humidity on mycelium composites

Determining the characteristic mechanical changes at dynamic temperature and humidity conditions is regarded as a powerful and sensitive "fingerprinting" tool, particularly for the characterization of polymer structures and complex porous materials [32]. Currently, DMA has not been extensively used to characterize mycelia composites, although it is commonly applied with many other materials.

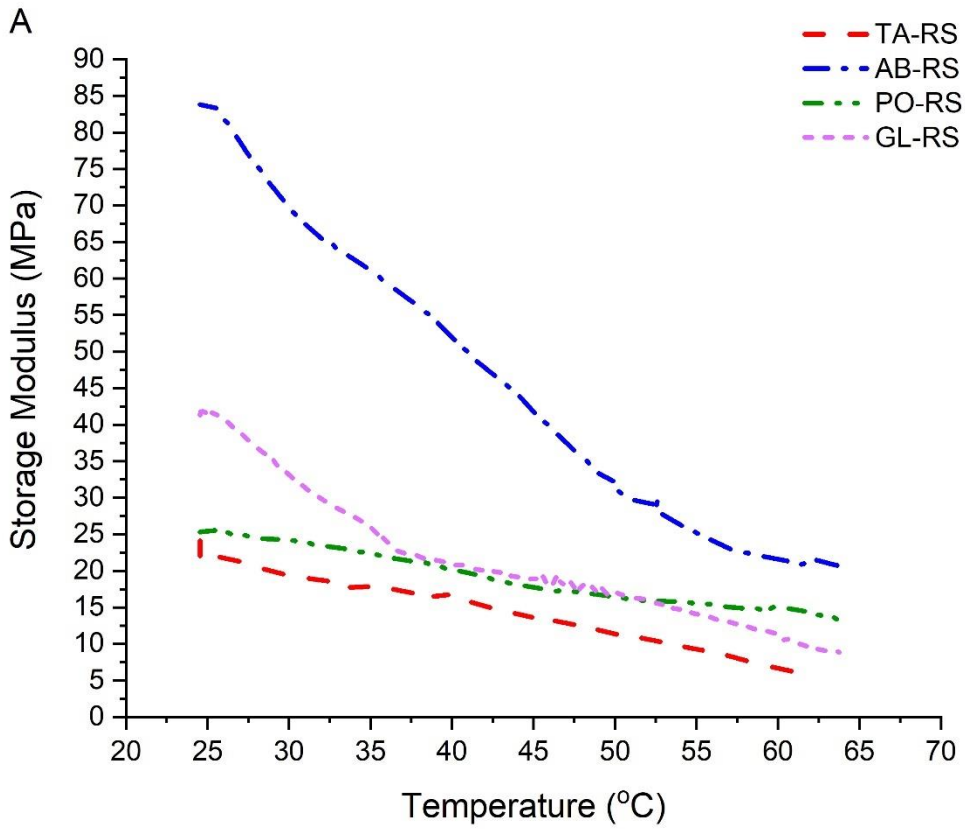
In the present study, RS-fed mycelia composites showed significantly higher values for storage modulus compared to OH-fed composites, both with changing temperature and RH. Selection of substrate affects the morphology and mechanical properties of the mycelium composites [25]. In polymer science, highly cross-linked thermoset polymers show much larger storage moduli than lightly cross linked ones, indicating that a tight network structure is related to high stiffness [3]. The intrinsic properties of biocomposite matrix components and the interfacial nature between the components govern the dynamic mechanical properties of composites [3, 44]. Although a high polysaccharide content has been correlated with stiff structure [32], the more rigid structure of RS substrates likely leads to stiffer mycelium composites compared to mycelia fed with OH.

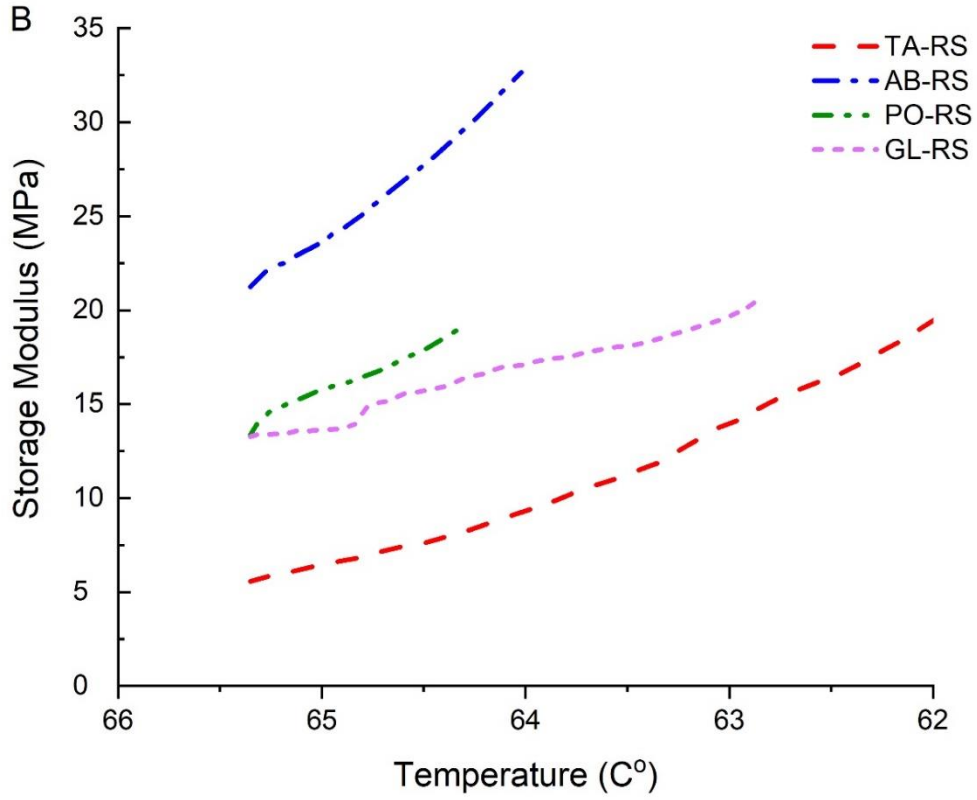
Figure 5 shows the storage modulus of RS-fed mycelium composites determined at 1 Hz frequency. All DMA tests were also conducted at 5 Hz frequency, which consistently resulted in slightly higher storage moduli (data not shown). The highest storage modulus value during the temperature scans was measured for AB-RS (83 MPa) (Figure 5A). The high mycelium concentration due to the high growth rate of AB-RS may have reduced the mobility and deformation of the material, leading to high stiffness [40]. RS seemed to provide better growth conditions than OH, which could enhance the hyphae morphology development.

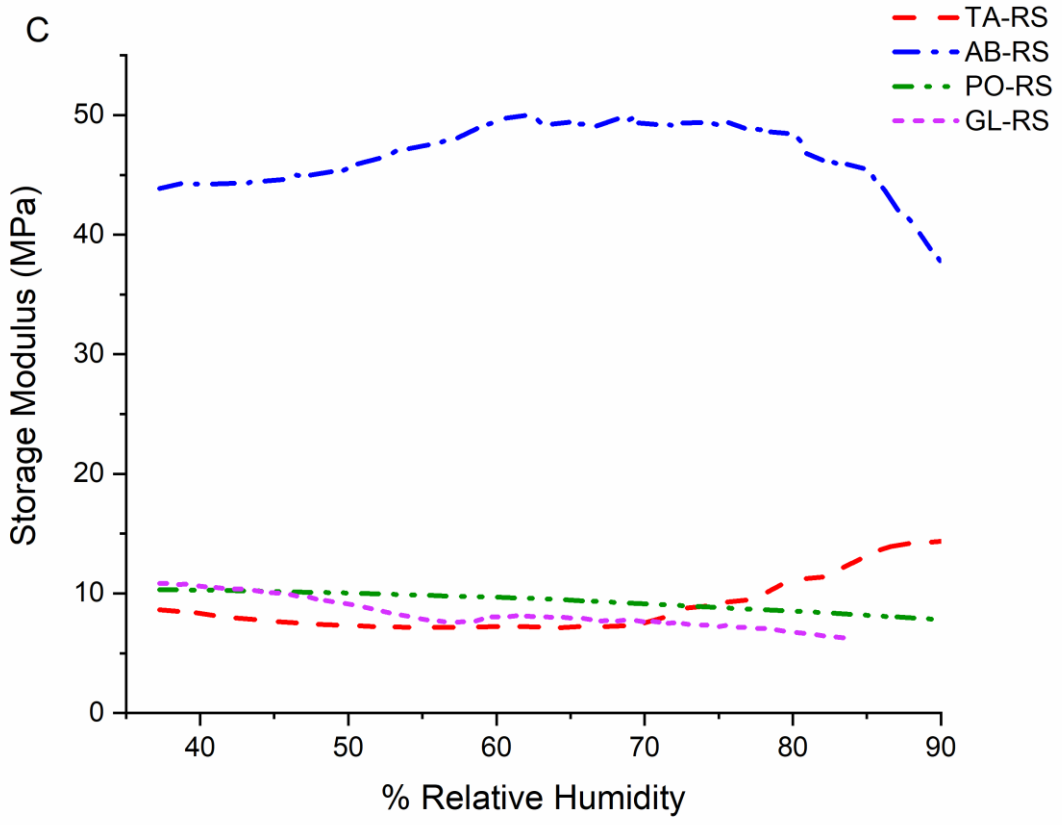
Increasing the temperature from 25ºC to 65ºC decreased the stiffness of all RS-fed mycelium composites. Even though the modulus of AB-RS composites decreased the most, they still exhibited the highest modulus at 65ºC compared to all studied mycelium composites. The AB-RS sample gave a lower compressive strength than the other mycelia composites (as discussed in Section 3.4). Together with the DMA measurements, this finding indicates that it had a unique elastic behavior.

AB-RS showed high density similar to that of TA-RS, however such similarity in their density did not reflect their mechanical behavior in the changing environmental conditions.

The storage moduli of the OH-fed mycelium composites varied between 0.32 and 0.87 MPa (data not shown) and were relatively low in comparison to those fed with RS (7–83 MPa). GL-OH gave an increasing storage modulus until 45ºC (0.76 MPa), which then dropped slightly under its initial storage modulus at 65ºC (0.48 MPa). This behavior was different from that of TA-OH and PO-OH, which showed a decreasing trend followed by an increase in strength with increasing temperature. For AB-OH, on the other hand, the storage modulus increased with temperature. Previously, *P. ostreatus* inoculation of wheat straw as a filler for high-density polyethylene production has given promising storage moduli results by improving interfacial adhesion [45]. However, determining the impacts of dynamic temperature and relative humidity conditions using DMA is a novel approach for mycelium composites.




B
Storage Modulus (MPa)
Temperature (C°)
TA-RS
AB-RS
PO-RS
GL-RS
0
5
10
15
20
25
30
35
66
65
64
63
62


C
Storage Modulus (MPa)
% Relative Humidity
TA-RS
AB-RS
PO-RS
GL-RS
0
10
20
30
40
50
40
50
60
70
80
90

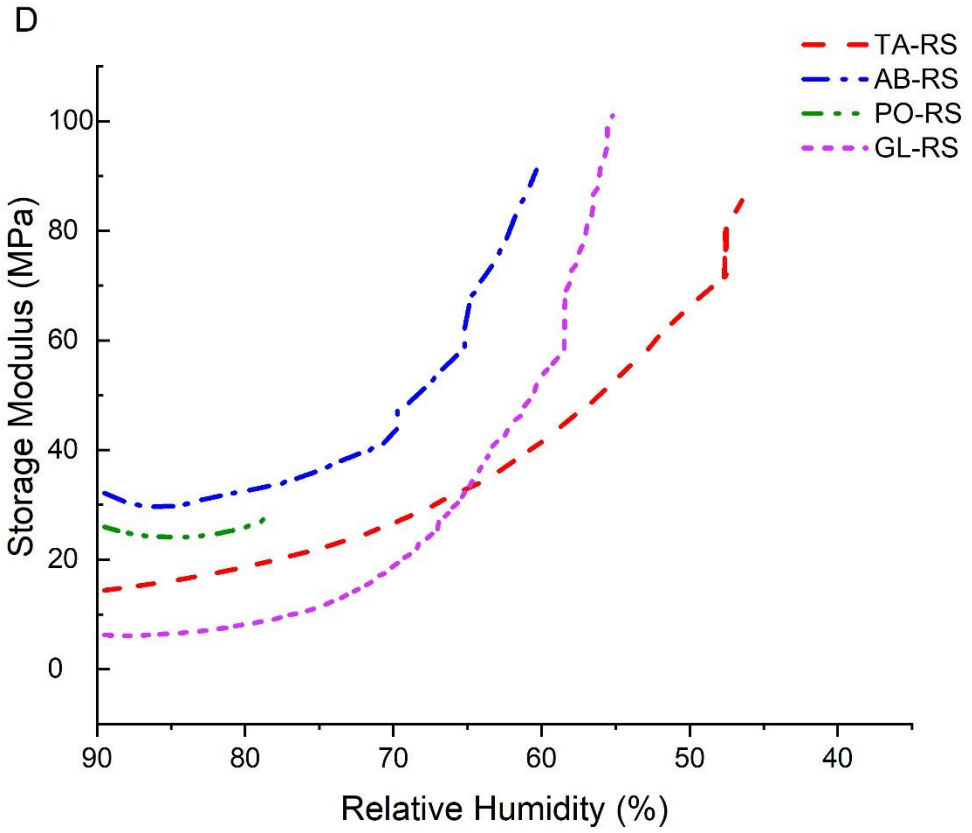


Figure 5. Storage moduli of mycelium composites

Rapeseed cake (RS)-fed mycelium composites at A) increasing temperature, B) decreasing temperature, C) increasing relative humidity, and D) decreasing relative humidity. TA: *Trichoderma asperellum*, AB: *Agaricus bisporus*, PO: *Pleurotus ostreatus*, and GL: *Ganoderma lucidum* mycelia.

A decrease in temperature after heating increased the storage modulus of all studied mycelia composites (Figure 5B). However, the extent of the stiffness increase depended on the feeding substrate and mycelia strain. Increasing stiffness caused brittleness in RS-fed mycelia; GL-RS collapsed during cooling at 62.7ºC, and TA-RS at 63ºC. The other studied RS-mycelium composites were more fragile and broke at higher temperatures. In contrast, the OH-fed mycelium composites showed increased storage moduli until the end of the analysis, down to 25ºC. The increase in stiffness was steep for GL-OH, while PO-OH only showed a minor increase. The behavior of TA-OH and AB-OH were between that of GL-OH and PO-OH. The mechanical properties of the composites generally display weakening at elevated temperatures, since polymers become pliable and lose their homogeneous behavior as a matrix element [46]. Moreover, high temperatures may cause the formation of microscopic cracks and voids in the composite material matrix, and hence reduce the storage modulus [44]. The mechanical behavior of mycelium

composites may differ from that of polymer matrices. For example, the behavior of mycelium composites is dominated by the mycelium matrix at low strains, whereas higher strains lead to the rapid stiffening due to the contact established between mycelia and feeding substrate [8]. Therefore, an increase in stiffness may be related to the mycelia hyphae acting as filler after the applied heat treatment, to limit the porous structure of the substrate and therefore enhancing the material stiffness, in a similar mechanism to brewer's spent grain used as fillers to limit the mobility of polymer chains [47]. The increased stiffness during cooling may be explained by heat-induced interaction, such as physical cross-linking between the functional groups of composite matrix elements, which may lead to an increase in stiffness [22].

Making humidity scans with DMA is an established method to characterize biopolymer materials, such as films from bacterial cellulose and glucuronoxylan [24], spruce galactoglucomannans [27], calcium caseinate [48], or hydrogels with carboxymethylcellulose [22]. Water plays a role as a plasticizer at high humidity for most polysaccharides due to the solubilization/plasticization phenomenon. Hence, an increase in RH generally causes softening of those materials [42]. The effect of hydrodynamic treatment on the studied RS-fed mycelium composites was different from the above mentioned materials. After the initial slight softening, AB-RS (until 50% RH) and GL (until 60% RH) became stiffer with increasing humidity (Figure 5C). In contrast, PO-RS demonstrated a constant softening. For AB-RS, a gradual increase in stiffness was seen until around 60% RH, followed by a rather constant storage modulus until 85% RH, at which point it softened. The change in storage modulus during the increasing RH was about 10 MPa. The significantly higher stiffness in AB-RS samples might also be related to the water uptake data discussed above, as a clear difference in water uptake of AB-RS samples was evident. Lower water uptake may have increased the stiffness at changing environmental conditions. However, AB-RS was discrete also in compressive strength, as all other RS-fed samples had higher strength (Fig 3). The effect of RH on storage modulus of OH-fed mycelia composites was minor, as the samples were significantly softer (0.1–0.90 MPa) than the RS-fed mycelia composites (results not shown). An increase in storage modulus after increasing the relative humidity has not been commonly observed, and generally a softening is expected [22, 49]. In mycelia-based materials, the presence of mycelia hyphae may serve as a hydrophobic reinforcement in the material structure [45].

After the DMA test with increased RH, another test with decreasing RH was performed. The decreasing RH further increased the storage modulus of all samples, regardless of the substrate (Fig. 5D). GL-RS gave the highest storage modulus value (by increasing from 5 MPa over 100 MPa), after the RH was decreased to 60%. TA-RS showed the increasing storage modulus further until 50% RH (85MPa). This RH range is close to the RH at which the mycelium composites showed the

first change in their hydromechanical characteristics during the initial RH increase, but with significantly increased storage modulus. An increase in stiffness was also evident for OH-fed mycelium composites, although the increases were generally smaller.

Mechanical properties of mycelium composites are mainly determined by cohesion between the fungal hyphae and polymer matrix of the substrate material. Hyphal differences among different fungal species, along with the differences in their substrate degradation mechanisms, play a key role in mechanical properties [32]. Although GL and PO are both lignocellulose degrading fungi, the morphological differences between these two strains – namely the higher flexibility of the twisted and branched structure of GL – have been previously related with their higher strength and elongation [25]. Moreover, binding hyphae type has been linked with higher material strength, whereas generative type of hyphae has been associated with a more limited mechanical strength [17]. In the present study, the novel mycelium composite of AB-RS showed an especially high mechanical stiffness and responded to successive humidity increase and decrease with increased stiffness.

4. Conclusion

We showed that of the nine known strains that were pre-screened and the two novel fungal strains for mycelium composites characterized, *A. bisporus* (AB) was especially stiff and strong compared to the previously known mycelia strains *P. ostreatus* (PO) and *G. lucidum* (GL). The dense structure and rich chemical composition of rapeseed cake made it a potent feeding substrate for mycelia. For the first time, we determined the dynamic mechanical properties of mycelium composites at a broad moisture gradient and showed that AB grown on rapeseed cake was resistant towards high humidity. Hydromechanical stress factors applied via DMA analysis worked as an efficient tool to simulate the possible conditions for mycelium composites during expected consumer usage. The fungal mycelium composites have high potential and capability to be used more widely in future material solutions due to their promising properties of adaptation to different ambient conditions, as well as sustainability, low cost, and ease of production.

CRediT authorship contribution statement

Zeynep Tacer Caba: Methodology development, Experimental work and data analysis, Writing: original draft, review & editing. **Jutta J. Varis:** Methodology development, Experimental work and data analysis, Writing: review & editing. **Pauliina Lankinen:** Experimental work and data analysis, Writing: review & editing. **Kirsi S. Mikkonen**: Conceptualization, Methodology development, Supervision, Writing: review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Appendix A. Supplementary data

The Supplementary Materials contain information on the FT-IR spectra of the mycelia both as plain and as grown on substrates, and stress-strain curves of mycelia determined with texture analysis.

References

[1] W. Chen, H. Hao, D. Hughes, Y. Shi, J. Cui, Z.-X. Li, Static and dynamic mechanical properties of expanded polystyrene, Mater. Design 69 (2015) 170-180.
[2] UNEP, Single-Use Plastics A Roadmap for Sustainability, UNEP (United Nations Environment Programme), 2018.
[3] N. Saba, M. Jawaid, O.Y. Alothman, M. Paridah, A review on dynamic mechanical properties of natural fibre reinforced polymer composites, Constr. Build. Mater. 106 (2016) 149-159.
[4] G.A. Holt, G. McIntyre, D. Flagg, E. Bayer, J.D. Wanjura, M.G. Pelletier, Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: Evaluation study of select blends of cotton byproducts, J. Biobased Mater. Bio. 6(4) (2012) 431-439.
[5] E.I. Saavedra Flores, M.I. Friswell, Y. Xia, Variable stiffness biological and bio-inspired materials, J. Intel. Mat. Syst. Str. 24(5) (2012) 529-540.
[6] F.V.W. Appels, J. Dijksterhuis, C.E. Lukasiewicz, K.M.B. Jansen, H.A.B. Wösten, P. Krijgsheld, Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material, Sci. Rep. 8(1) (2018) 4703.
[7] L. Jiang, D. Walczyk, G. McIntyre, W.K. Chan, Cost modeling and optimization of a manufacturing system for mycelium-based biocomposite parts, J. Manuf. Syst. 41 (2016) 8-20.
[8] M.R. Islam, G. Tudryn, R. Bucinell, L. Schadler, R.C. Picu, Mechanical behavior of mycelium-based particulate composites, J. Mater. Sci. 53(24) (2018) 16371-16382.
[9] J.L. Teixeira, M.P. Matos, B.L. Nascimento, S. Griza, F.S.R. Holanda, R.H. Marino, Production and mechanical evaluation of biodegradable composites by white rot fungi Ciência e Agrotec. 42 (2018) 676-684.
[10] M. Jones, A. Mautner, S. Luenco, A. Bismarck, S. John, Engineered mycelium composite construction materials from fungal biorefineries: A critical review, Mater. Design (2019) 108397.
[11] L. Jiang, D. Walczyk, G. McIntyre, R. Bucinell, G. Tudryn, Manufacturing of biocomposite sandwich structures using mycelium-bound cores and preforms, J. Manuf. Syst. 28 (2017) 50-59.
[12] G.J. Tudryn, L.C. Smith, J. Freitag, R. Bucinell, L.S. Schadler, Processing and morphology impacts on mechanical properties of fungal based biopolymer composites, J. Polym. Environ. 26(4) (2017) 1473-1483.

[13] S. Karimi, N. Mahboobi Soofiani, A. Mahboubi, M. Taherzadeh, Use of organic wastes and industrial by-products to produce filamentous fungi with potential as aqua-feed ingredients, Sustainability 10(9) (2018) 1-19.
[14] R. Mazur, Mechanical Properties of Sheets Comprised of Mycelium: A Paper Engineering Perspective, Environmental Resources Engineering Department, State University of New York, College of Environmental Science and Forestry., 2015.
[15] S. Travaglini, J. Noble, P. Ross, C. Dharan, Mycology matrix composites, Proceedings of the American Society for Composites–28th Technical Conference, State College, PA, Sept. Sept, 2013, pp. 1-20.
[16] J.A. López Nava, J. Méndez González, X. Ruelas Chacón, J.A. Nájera Luna, Assessment of edible fungi and films bio-based material simulating expanded polystyrene, Mater. Manuf. Process. 31(8) (2016) 1085-1090.
[17] M. Jones, T. Huynh, C. Dekiwadia, F. Daver, S. John, Mycelium composites: A review of engineering characteristics and growth kinetics, J. Bionano. 11(4) (2017) 241-257.
[18] R.C. Correa, A.H. de Souza, R.C. Calhelha, L. Barros, J. Glamoclija, M. Sokovic, R.M. Peralta, A. Bracht, I.C. Ferreira, Bioactive formulations prepared from fruiting bodies and submerged culture mycelia of the Brazilian edible mushroom Pleurotus ostreatoroseus Singer, Food Funct. 6(7) (2015) 2155-64.
[19] M. Blackwell, The Fungi: 1, 2, 3… 5.1 million species?, Am. J. Bot. 98(3) (2011) 426-438.
[20] M.G. Pelletier, G.A. Holt, J.D. Wanjura, A.J. Lara, A. Tapia-Carillo, G. McIntyre, E. Bayer, An evaluation study of pressure-compressed acoustic absorbers grown on agricultural by-products, Ind. Crop. Prod. 95 (2017) 342-347.
[21] H.A. Wösten, Filamentous fungi for the production of enzymes, chemicals and materials, Curr. Opin. Biotech. 59 (2019) 65-70.
[22] A. Gregorova, N. Saha, T. Kitano, P. Saha, Hydrothermal effect and mechanical stress properties of carboxymethylcellulose based hydrogel food packaging, Carbohyd. Polym. 117 (2015) 559-568.
[23] M. Babaee, M. Jonoobi, Y. Hamzeh, A. Ashori, Biodegradability and mechanical properties of reinforced starch nanocomposites using cellulose nanofibers, Carbohyd. Polym. 132 (2015) 1-8.
[24] S. Dammström, L. Salmén, P. Gatenholm, The effect of moisture on the dynamical mechanical properties of bacterial cellulose/glucuronoxylan nanocomposites, Polymer 46(23) (2005) 10364-10371.
[25] M. Haneef, L. Ceseracciu, C. Canale, I.S. Bayer, J.A. Heredia-Guerrero, A. Athanassiou, Advanced materials from fungal mycelium: Fabrication and tuning of physical properties, Sci. Rep. 7(41292) (2017) 1-11.
[26] T.J. White, T. Bruns, S. Lee, J. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: D.G. MA Innis, JJ Sninsky, and TJ White (Ed.), PCR Protocols: A Guide to Methods and Applications1990, pp. 315-322.
[27] K.S. Mikkonen, M.I. Heikkilä, S.M. Willför, M. Tenkanen, Films from glyoxal-crosslinked spruce galactoglucomannans plasticized with sorbitol, Int. J. Polym. Sci. 2012 (2012) 1-12.
[28] K. Vega, M. Kalkum, Chitin, chitinase responses, and invasive fungal infections, Int. J. Microb. 2011 (2012) 1-10.
[29] S.M. Bowman, S.J. Free, The structure and synthesis of the fungal cell wall, Bioessays 28(8) (2006) 799-808.
[30] R. Pena, C. Lang, A. Naumann, A. Polle, Ectomycorrhizal identification in environmental samples of tree roots by Fourier-transform infrared (FTIR) spectroscopy, Front. Plant Sci. 5(229) (2014) 1-9.
[31] R.S. Lakes, Composite biomaterials, in: J.B. Park;, J.D. Bronzino (Eds.), Biomaterials: Principles and Applications, CRC Press, , Boca Raton, FL, 2003, pp. 79-80.

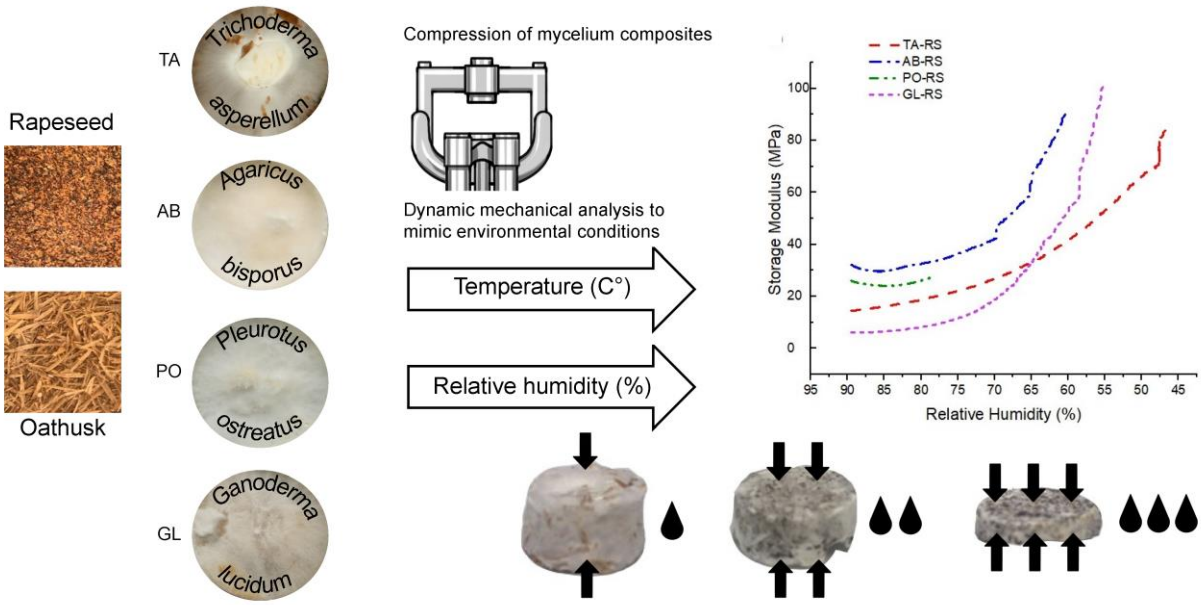
[32] C. Girometta, A.M. Picco, R.M. Baiguera, D. Dondi, S. Babbini, M. Cartabia, M. Pellegrini, E. Savino, , Physico-mechanical and thermodynamic properties of mycelium-based biocomposites: A review, Sustainability 11(1) (2019) 281-303.
[33] M.C.N.d. Andrade, M.T.D.A. Minhoni, L.B.D. Aguiar, C.S.M. de Carvalho, C. Sales-Campos, Uso de resíduos madeireiros da Amazônia brasileira no cultivo in vitro de Lentinus strigosus/Use of wood waste from the Brazilian Amazon in the in vitro cultivation of Lentinus strigosus, Ambiência 9(1) (2013) 189-196.
[34] J.W. King, G.R. List, Supercritical Fluid Technology in Oil and Lipid Chemistry, The American Oil Chemists Society1996.
[35] E. Çulcuoğlu, E. Ünay, F. Karaosmanoğlu, Rapeseed cake as a biomass source, Energ. Source. 24(4) (2002) 329-336.
[36] L.D. Varanda, M.F.d. Nascimento, A.L. Christoforo, D.A.L. Silva, F.A.R. Lahr, Oat hulls as addition to high density panels production, J. Mater. Res. 16 (2013) 1355-1361.
[37] M.R. Islam, G. Tudryn, R. Bucinell, L. Schadler, R.C. Picu, Morphology and mechanics of fungal mycelium, Sci. Rep. 7(1) (2017) 1-12.
[38] P. Amstislavski, Z. Yang, M.D. White, Thermal Insulation Material from Mycelium and Forestry Byproducts, Google Patents, 2017.
[39] J.A. López Nava, J. Méndez González, X. Ruelas Chacón, J.A. Nájera Luna, Assessment of edible fungi and films bio-based material simulating expanded polystyrene, Mater. Manuf. Process. 31(8) (2015) 1085-1090.
[40] Z. Yang, F. Zhang, B. Still, M. White, P. Amstislavski, Physical and mechanical properties of fungal mycelium-based biofoam, J. Mater. Civil Eng. 29(7) (2017) 04017-04030.
[41] B.K. Stefanowski, S.F. Curling, G.A. Ormondroyd, A rapid screening method to determine the susceptibility of bio-based construction and insulation products to mould growth, Int. Biodeter. Biodegr. 116 (2017) 124-132.
[42] J.J. Kochumalayil, L.A. Berglund, Water-soluble hemicelluloses for high humidity applications–enzymatic modification of xyloglucan for mechanical and oxygen barrier properties, Green Chem. 16(4) (2014) 1904-1910.
[43] F.V. Appels, S. Camere, M. Montalti, E. Karana, K.M. Jansen, J. Dijksterhuis, P. Krijgsheld, H.A. Wösten, Fabrication factors influencing mechanical, moisture-and water-related properties of mycelium-based composites, Mater. Design 161 (2019) 64-71.
[44] L.A. Pothan, Z. Oommen, S. Thomas, Dynamic mechanical analysis of banana fiber reinforced polyester composites, Compos. Sci. Technol. 63(2) (2003) 283-293.
[45] A. Schirp, F. Loge, S. Aust, P. Swaner, G. Turner, M. Wolcott, Production and characterization of natural fiber-reinforced thermoplastic composites using wheat straw modified with the fungus Pleurotus ostreatus, J. Appl. Polym. Sci. 102(6) (2006) 5191-5201.
[46] W. Al Azzawi, M.M. Islam, J. Leng, F. Li, J.A. Epaarachchi, Quantitative and qualitative analyses of mechanical behavior and dimensional stability of styrene-based shape memory composites, J. Intel. Mat. Syst. Str. 28(20) (2017) 3115-3126.
[47] A. Hejna, K. Formela, M.R. Saeb, Processing, mechanical and thermal behavior assessments of polycaprolactone/agricultural wastes biocomposites, Ind. Crop. Prod. 76 (2015) 725-733.
[48] L. Bonnaillie, P. Tomasula, Application of humidity-controlled dynamic mechanical analysis (DMA-RH) to moisture-sensitive edible casein films for use in food packaging, Polymers 7(1) (2015) 91-114.
[49] K.S. Mikkonen, M. Tenkanen, Sustainable food-packaging materials based on future biorefinery products: Xylans and mannans, Trends. Food Sci. Tech. 28(2) (2012) 90-102.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Zeynep Tacer Caba: Methodology development, Experimental work and data analysis, Writing: original draft, review & editing. **Jutta J. Varis:** Methodology development, Experimental work and data analysis, Writing: review & editing. **Pauliina Lankinen:** Experimental work and data analysis, Writing: review & editing. **Kirsi S. Mikkonen**: Conceptualization, Methodology development, Supervision, Writing: review & editing.



Research Highlights

- The dynamic mechanical properties of mycelium composites were studied for the first time at a broad moisture gradient.
- Novel mycelium composites from *Agaricus bisporus* gave high moisture-resistance.
- The dense structure and rich chemical composition of rapeseed cake made it a potent feeding substrate for mycelia.