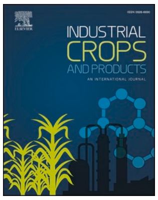
Industrial Crops & Products 170 (2021) 113742

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Valorization of wheat bran agro-industrial byproduct as an upgrading filler for mycelium-based composite materials

Laura Sisti [a], Claudio Gioia [a,*], Grazia Totaro [a], Steven Verstichel [b], Marco Cartabia [c,d], Serena Camere [d], Annamaria Celli [a]

[a] *Department of Civil, Chemical, Environmental and Materials Engineering, University of Bologna, Via Terracini 28, 40131, Bologna, Italy*
[b] *OWS nv, Dok Noord 5, 9000, Gent, Belgium*
[c] *Department of Earth and Environmental Sciences (DSTA), University of Pavia, 27100, Pavia, Italy*
[d] *MOGU S.r.l., Via San Francesco d'Assisi 62, 21020, Inarzo, VA, Italy*

ARTICLE INFO

Keywords:
Bran
Mycelium
Circular economy
Biorefinery
Structure/property relationship
Compostability

ABSTRACT

When considered by a biorefinery approach, an agroindustrial byproduct such as wheat bran can find a new standing in the field of fabrication of mycelium-based materials. The present work reports on a systematic study on the effect of wheat bran as an upgrading feedstock for the growth and development of fully biobased and biodegradable composites. Two families of materials based on bran/cotton and bran/hemp mixtures were fabricated on an industrial scale. The natural materials thus obtained were fully characterized and their end-life was assessed in composting conditions. The research focusses on two main aspects: the nutritional contribution of bran for the fungal growth and its effect on the mechanical properties as a filler in the final composites. It must be noted that the valorization and exploitation of a byproduct such as bran can have a considerable impact on the industrial production of mycelium-based composite materials, by reducing the time of production while increasing their mechanical performances.

1. Introduction

The advent of green chemistry and the development of social awareness about sustainability that mark our times are the first welcome answers to the overwhelming impact of human activities on the environment. Understanding that manufacturing and agro-industrial activities have up to now privileged mass production rather than the correct equilibrium with natural resources, constitutes the first step of an unprecedented shift toward concepts such as circular economy and biorefinery.

Circular economy aims to tackle social, economic and environmental concerns by proposing a shift from a pattern of linear economy, defined by the direct disposal of post-consumer goods, to multiple loops of exploitation, based on reuse, recovery and recycle.(Ghisellini et al., 2016) A further step in the direction of sustainability is perfectly epitomized by circular bio-economy which joins a circular approach with a responsible exploitation of biomass sources.(D'Amato et al., 2017; Sherwood, 2020; Pinales-Márquez et al., 2021)

Such an ambitious challenge is based on the concept of biorefinery which is defined as the rational design of the exploitation of biobased resources in order to maximize the production of valuable goods while minimizing waste.(Londo et al., 2018; Dahmen et al., 2019; Ubando et al., 2020) As a consequence, waste valorization plays a most relevant role, as it can be the discriminating factor between a biorefinery success or its failure. When it is well-planned, the availability of waste will find new exploitations for several novel applications, thus becoming a valuable byproduct.(Fritsch et al., 2017)

A relevant example is given by the current advances in cereal biorefinery.(Elmekawy et al., 2013) World cereal production has increased to 2742 million tons in 2020,(Prospects and Situation, 2020) resulting in the connected increase of wheat bran, rice husk, sweet sorghum bagasse, corn dry distillers grain, corn cob and brewer's spent grains. Among all, bran deriving from corn, rice, and wheat biorefineries, is the most important milling byproduct.(Chandrasekaran, 2012)

Two main strategies concern bran valorization in materials science. The first employs it as a feedstock to recover valuable building blocks such as hemicellulosic derivatives, starch, lactic acid, polyphenols, ferulic acid and proteins.(Apprich et al., 2014; Ruthes et al., 2020;

---

* Corresponding author.

E-mail addresses: laura.sisti@unibo.it (L. Sisti), claudio.gioia2@unibo.it (C. Gioia), steven.verstichel@ows.be (S. Verstichel), mc@mogu.bio (M. Cartabia), sc@mogu.bio (S. Camere), annamaria.celli@unibo.it (A. Celli).

https://doi.org/10.1016/j.indcrop.2021.113742
Received 1 March 2021; Received in revised form 10 June 2021; Accepted 11 June 2021

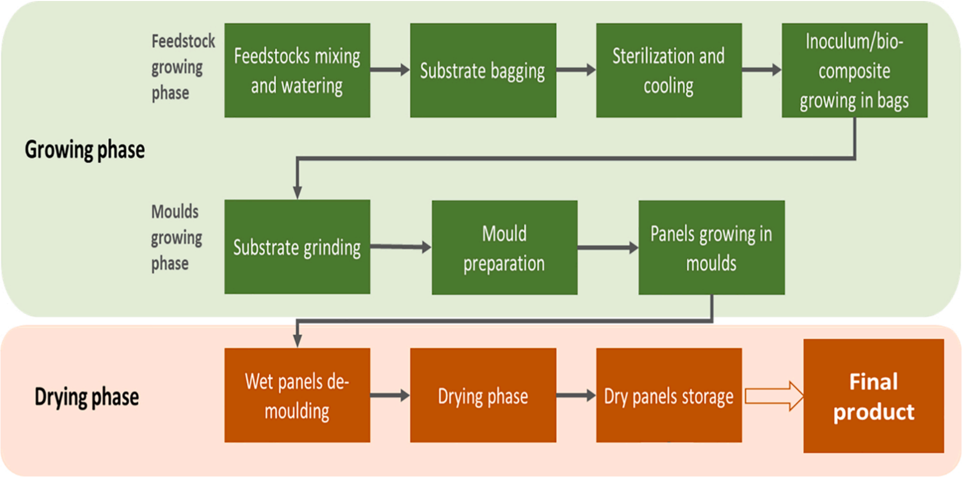


Fig. 1. Industrial procedure for the fabrication of mycelium-based composites.

Germec et al., 2019) In this case, its structural architecture is degraded by physical, chemical and microbiological processes to produce target compounds. However, such an approach requires the set-up of several equipment units devoted to the pretreatment of the feedstock, its controlled degradation (sometimes requiring multiple steps) and the separation of the value-added products from a mixture of compounds. As a consequence, large investments and an accurate process design is required to reach a suitable market scale.(Rudjito et al., 2019) The Agrimax project remarkably embodies this approach by designing and building pilot plants able to convert their equipment to maximize the recovery of key biobased architectures from different natural feedstocks.(Anon, 2021"https://agromax.iris.cat/")

The second strategy uses unmodified bran as a reinforcement/filler for polymeric materials to fabricate bio-composites.(Shankar et al., 2014; Rahman et al., 2017; Gigante et al., 2020; Formela et al., 2016; Pan et al., 2006) In this regard, bran can be considered a cheap biodegradable reinforcing agent, which could lower the price of the polymer matrices and modulate their mechanical properties. Such an approach is relatively easy to apply and to scale-up on an industrial scale. In this case, the main limitation concerns the thermal and mechanical stability of bran in the processing conditions. As a matter of fact, high temperatures of execution can induce a partial degradation of the lignocellulosic matrix resulting in undesired discoloration and smell. Moreover, poor adhesion between polymeric hydrophobic matrix and hydrophilic fibers is observed and the result affects the mechanical performances of the composite.(Gigante et al., 2020) Several petrol based composites were recently produced with this method involving polymers such as polyethylene,(Hejna et al., 2020) polypropylene(Sohn et al., 2019) and styrene butadiene rubber and also bio-based polymers, such poly(hydroxy alkanoate)s.(Zhang et al., 2017) In this case, the sustainable character of the composite is completely dependent on the contribution of the polymeric matrix.

The present work deals with a third strategy concerning the development of highly sustainable (Jones et al., 2019a) composites deriving from fungal mycelium. They make up a new class of environmentally friendly materials since they are created by networks of filamentous fungi hyphae grown over low-cost organic wastes.(Liu et al., 2019; Jones et al., 2017; Girometta et al., 2019; Pelletier et al., 2013; Joshi et al., 2020; Antinori et al., 2020; Jones et al., 2018a, 2018b, 2019a, 2019b) Even though they feature properties often comparable with technical grade polymeric foams, they are 100 % made of natural and biodegradable compounds obtained through inexpensive biological processes rather than energy-consuming manufacturing methods. Their potential industrial applications include packaging and construction materials, acoustic dampers, super absorbents and electronic parts.,(Abhijith et al., 2018) The mechanical properties of this class of biological composites are influenced by parameters such as the elasticity, branching and network density of the mycelium filaments, as well as by the contribution of the natural matrixes fed to the fungus.(Islam et al., 2017; Haneef et al., 2017; Elsacker et al., 2019; Nawawi et al., 2020) The possibility to modulate their surface hydrophilicity is currently opening new possibilities in challenging fields, such as microfluidics, smart coatings, and self-cleaning surfaces.(Jones et al., 2019a; Sun et al., 2021) Although literature currently reports examples of mycelium-based materials fabricated on a laboratory scale by employing lignocellulosic feedstocks such as cotton, hemp, flax, straw,(Elsacker et al., 2019) wood and cellulose,(Sun et al., 2019) a comprehensive understanding of the full potential of this class of materials is far from complete, especially when an industrial scale is taken into consideration.

Within this scenario, the exploitation of bran is very attractive and currently completely unexplored. On one side, bran can act as a cheap lignocellulosic feedstock finding a new role in the growth of the mycelium fibers; on the other side, it can contribute as a filler/reinforcing agent to the thermo-mechanical properties of the resulting composites.

Reported here is the industrial scale fabrication of mycelium materials based on the combination of cotton, hemp and wheat bran on both a laboratory and an industrial scale. The purpose of the present work is to explore the range of performances of such materials, obtained by industrial optimized processes, and to outline the specific contribution of wheat bran. Such materials were fully characterized to determine their morphology, surface and mechanical properties. Compostability was finally estimated to assess and understand the behavior of such materials at their end-life.

2. Materials and methods

2.1. Materials

Mycelium inoculum is grown by Mogu from a wood decay basidiomycete strain (proprietary information of Mogu, Lombardy, Italy). Non-woven low-quality cotton is obtained from the residues of the production of cotton yarning (third grade residues) and presents fibers up to 2 cm long. Hemp shives are obtained as a byproduct from the production of hemp fibers, presenting the following dimension ranges: width 3−6 mm, length 10−15 mm, thickness 1−1,5 mm. Wheat bran is supplied by Barilla and it is composed by irregular flakes of about 1 mm^3.

2.2. Fabrication procedure of the mycelium-based composites

The synthetic protocol used on an industrial scale to obtain the

Table 1
starting composition of the mycelium-based materials.

	Sample Name	Wheat Bran (%)	Growth Rate (mm/days)
Hemp	HB0	0	8.9
	HB10	10	17.8
	HB20	20	17.8
	HB30	30	17.8
Cotton	CB0	0	8.9
	CB10	10	17.8
	CB20	20	17.8
	CB30	30	17.8

mycelium composites was adapted from previously reported procedures.(Appels et al., 2019) Fig. 1 shows the two phases of the biocomposite production, namely the growing and the drying phases, and the related steps adopted for the fabrication of the mycelium-based materials on an industrial scale.

The feedstocks (bran cotton and hemp) were firstly taken from compressed bales and mixed together with hot water for 20 min in order to obtain a homogenised mass at 60 % of relative humidity. After this operation, the bulk fiber was then placed in polypropylene thermal resistant and microfiltered SacO2 bags (PP50/SEU4/V40−51) that allow the next steps, more specifically the conditions of humidity, pH, and gas exchange. Bags were then closed with tape and sterilized for 90 min at 123 °C at 1500 bar in order to kill all bacterial and fungal spores already present in the humid biomass. After cooling till reaching a proper temperature, the fungal strain (5%) was inoculated following an aseptic procedure in a laminar flow cabin. The colonization was assessed empirically by observing the macroscopic mycelium growth through the sealed bags. The growth rate was determined by measuring the time occurred to the mycelium to reach the bottom of the sealed bag from the top. Once reached a homogeneous colonization, the material is ready for the subsequent phases of product manufacturing. The colonized substrate was therefore removed from the bag, ground to a particle size of approximately 5−8 mm, and placed into a plastic thermo-formed mould. The material was gently hand-pressed to distribute the substrate as uniformly as possible and covered with perforated cellophane foil (0.35 μm). The fungus is allowed to grow further in a growing room with 75 % of humidity and at 25 °C for 14 days in the dark, achieving the physical status of a wet bio-panel. Finally, the panels are dried for 24 h at 50 °C to deactivate the mycelium. Through such drying technique, a biologically stable dry panel can be produced.

As shown in Table 1, two families of materials were fabricated according to the reported protocol. HBx and CBx indicate the materials based on hemp/bran and cotton/bran mixtures respectively, while the x stands for the bran weight percentage used in the feed.

2.3. Characterization

2.3.1. SEM analysis

Fragments of the skin of mycelium-based composites were collected and introduced in a variable pressure chamber of a scanning electron microscope (SEM) “Zeiss EVO 50 EP” equipped with a microprobe EDX “Oxford INCA 350″ and observed at different magnifications.

2.3.2. ATR-FTIR analysis

The analysis was conducted over the wavenumber range 650 – 4000 cm^{-1} using a PerkinElmer Spectrum One spectrometer equipped with a Universal ATR sampling accessory. 16 scans were taken for each spectrum at a resolution of 4 cm^{-1}. In case of composite materials, the measurements were carried out on at least 3 inner random points of each composite sample, in order to have a representative spectrum of all measurements. The starting materials, as well as the mycelium from the surface were also analyzed.

2.3.3. Static contact angle measurements

All samples were stored under vacuum, at room temperature, for 40 h, before measurements. The water contact angles were measured using a drop shape analyzer (DSA30S- Kruss) and recorded immediately after the water drop deposition. The measurements were stable in time. The results are expressed as average of at least 7 measurements, taken on the smooth surface of mycelium.

2.3.4. Mechanical characterization

All samples were characterized by flexural and compression tests after drying under vacuum for one night, according to an adapted procedure reported as ISO 1209 and ISO 844 respectively using an INSTRON 5966 series test instrument, equipped with a 10 kN load cell.

For flexural tests, five specimens (120 × 20 × 30 mm) for each sample were analyzed at room temperature and 70 ± 10 % R.H. at a constant rate of 10 mm/min and clamp support distance of 60 mm.

For compression tests, five specimens (100 × 100 × 50 mm) for each sample were analyzed at room temperature and 70 ± 10 % R.H. at a constant rate of 5 mm/min. The contact surface of the plates was not perfect due to the rough surface of the samples. The compression strength was obtained from the stress-strain relationships for each sample at a fixed strain since no peak occurred in the stress-strain relationship (strain-hardening behavior).

2.3.5. Compostability test

The industrial compostability was evaluated in line with the harmonized European standard EN 13,432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000). This standard defines 4 requirements: (1) material characteristics (organic matter content > 50 % on total solids and absence of heavy metals and

Fig. 2. Main lignocellulosic materials used on the production trials: A) Cotton, B) Hemp shives, C) Wheat bran.

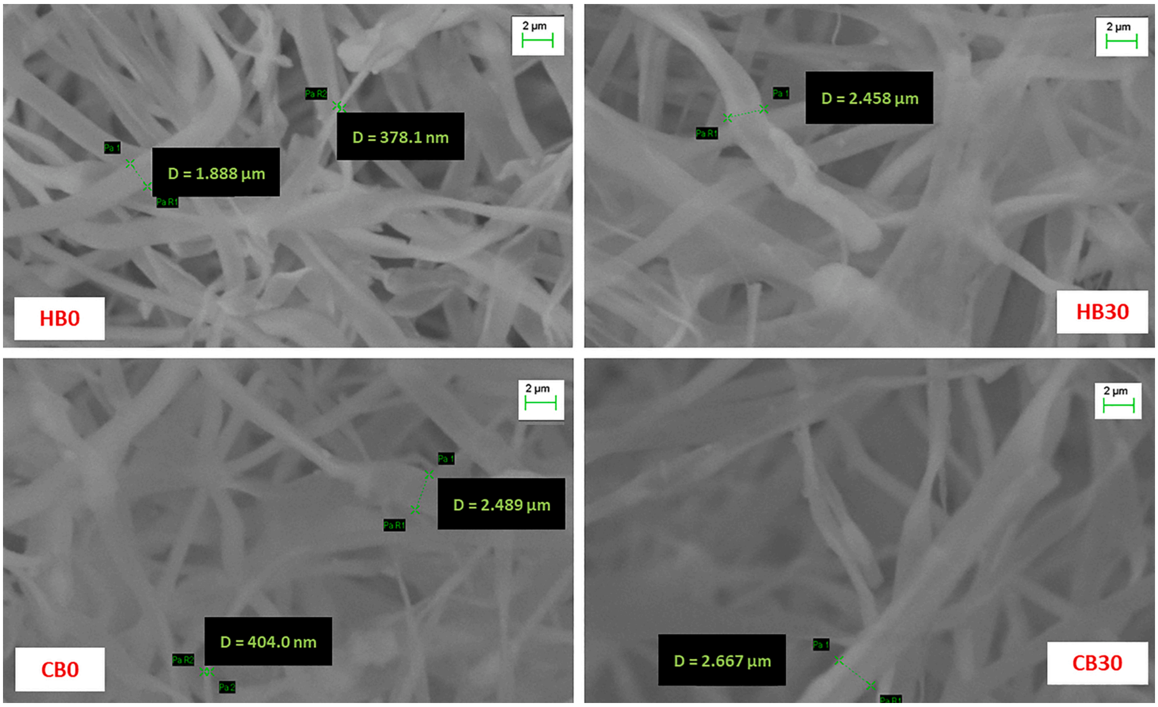


Fig. 3. External aspect of the final hemp and cotton panels with 30 % wt bran content and SEM analysis of the outer layer.

fluorine), (2) biodegradation, (3) disintegration and (4) no negative effect on compost quality. As the material is produced from natural lignocellulosic materials by a natural process, it is mainly the disintegration during composting that needs to be demonstrated. Therefore, the disintegration was qualitatively evaluated in a pilot-scale composting test based on ISO 16,929 Plastics – Determination of the Degree of Disintegration of Plastics Materials under Defined Composting Conditions in a Pilot-Scale Test.

3. Results and discussions

3.1. Fabrication of the composites

Among different lignocellulosic feedstocks, cotton and hemp shives were specifically chosen as main substrates for the fabrication of mycelium-based materials since both of them consist in non-food industrial crops and constitute a well-known substrate for mycelium growth (Fig. 2).

The cotton fraction used is a high-volume and low-cost waste stream derived from the cotton processing industry and constituted by very short fibers (<1.5 cm), lignin and dust. Hemp is considered as a low-maintenance crop and is a valuable low input alternative in intensive cropping systems as well as a profitable part of a rotation in extensive systems. It has relatively low fertilizer requirements, it provides excellent weed control and it does not require pesticides. The role of these feedstocks in the composite is to provide the main supporting substrate for fungal growth. Wheat bran, supplied by Barilla, is a residue of the company's industrial production and therefore is not suitable for human nutrition. Hence, the production of composites is clearly not in competition with the food chain.

The synthetic procedure previously reported was optimized on an industrial scale for the fabrication of two different lines of materials obtained using cotton/bran and hemp/bran mixtures (Table 1). The amount of wheat bran used ranged from 0 to 30 wt% since higher concentrations induced structural instabilities in the final material.

The presence of bran in the feedstock composition showed an obvious effect on the fungal growth during the first phase. While control samples such as CB0 and HB0 are fully colonized in 4 weeks with a growth rate of 8.9 mm/day, the presence of bran, regardless of the concentration, can induce an impressive boost in the growth speed thus reducing the step time and increasing the growth rate up to 17.8 mm/day. Such an effect is due to the rich nutrients composition that bran confers to the mixture. The presence of easily metabolizable compounds such as carbohydrates and proteins in bran, enhances fungal division at the initial growth stages while minerals and micronutrients contribute to fungal growth. Specifically, magnesium supports enzyme activity, cell and organelle structures; phosphorous is involved in the biosynthesis of nucleic acids, phospholipids and glycophosphates; potassium supports ionic balance and enzyme activity and sulphur is a source of sulphydryl amino acids and vitamins.(Kavanagh, 2005)

3.2. SEM analysis

The effect of bran on the morphology of the external surface of the composites was assessed by comparing the specimen obtained with 30 wt% of bran (CB30 and HB30) with the ones obtained in its absence (CB0 and HB0). As a matter of fact, the mycelium forms a skin-like layer at the substrate-air interface during the phase growth on the molds that binds the entire bulk and confers dimensional integrity to the artifact.

As highlighted in Fig. 3, and extensively reported in Figures A1-A4, all the samples exhibit a uniform outer layer entirely made of string-shaped filamentous hyphae. The mycelium fibers have similar morphology, with a width ranging between 1.8 and 2.7 μm and a thickness of about 400−690 nm. Although mycelium materials containing wheat bran tend to display larger hyphae (2.5/2.7 μm width), no obvious impact of the feed composition appears in the samples.

3.3. FTIR analysis

The chemical nature of the materials was evaluated by FTIR spectroscopy. ATR-FTIR spectra of the samples summarized in Table 1 are reported in Fig. 4 along with the reference agroindustrial feedstocks (hemp, cotton and bran), and the mycelium from the surface.

Hemp fibers, employed as reference material, are reported to contain

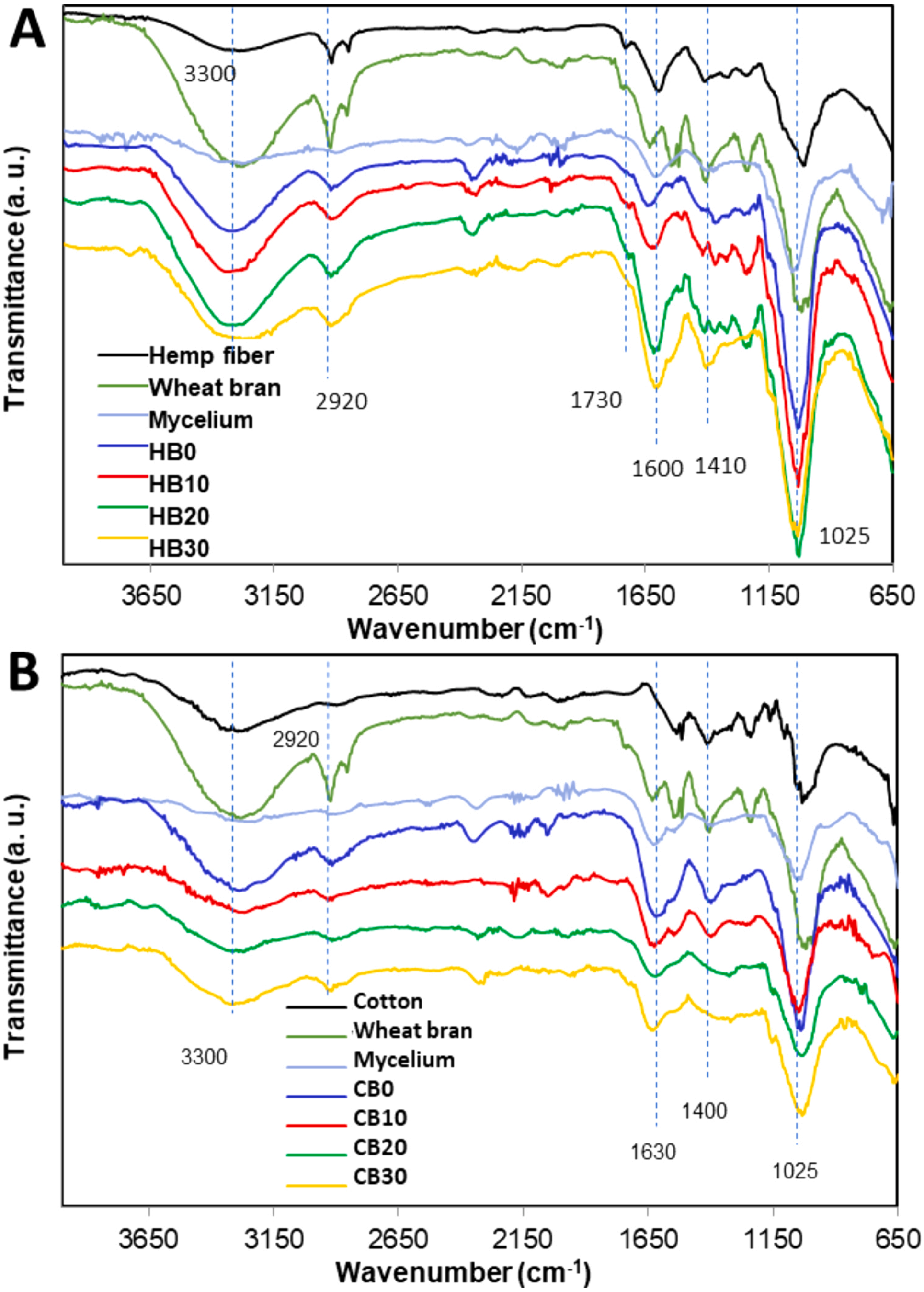


Fig. 4. ATR-FTIR spectra of the mycelium composites based on A) Hemp/Bran mixtures; B) Cotton/Bran mixtures.

cellulose, hemicellulose, waxes, oils, lignin and pectin.(Sisti et al., 2016) Mature cotton fibers are composed mostly of cellulose, followed by proteins, waxes, pectins, inorganics and other substances, such as lignin. (Liu and Kim, 2017) Wheat bran is reported to be composed of mainly dietary fiber (xylans, lignin, cellulose, galactan, fructans). Other components include vitamins, minerals and bioactive compounds such as alkylresorcinols, ferulic acid, flavonoids, carotenoids, lignans and sterols.(Wang et al., 2009; Onipe et al., 2015) The self-grown mycelium on the other hand contains lipids, proteins, nucleic acids (phosphate, sugar, nitrogenous bases) and polysaccharides.(Leung et al., 2006)

In detail, in Fig. 4A, hemp fiber presents the typical signal of OH stretching of polysaccharides (from cellulose and hemicellulose) near 3300 cm^{-1} and the CH stretching at 2850 cm^{-1}(waxes and oils). The signal at 1730 cm^{-1} (carbonyl stretching of acetyl group in hemicelluloses and methyl ester in pectin) and the signals at 1420 cm^{-1}, 1516 cm^{-1} and at 1250 cm^{-1} (aromatic rings of lignin) are also visible. The typical cellulose bands, due to glycosidic bond and pyranoid ring, can be observed at 850 cm^{-1}. Wheat bran profile shows the hydroxyls from carbohydrate and phenolic groups (3600 – 3200 cm^{-1}), aromatic rings and/or double bonds (1550 – 1200 cm^{-1}) from bioactive molecules, C—O, C—C, C—O—C vibrations from polysaccharides (1200 – 900 cm^{-1}). The self-grown mycelium presents amide I, II, III bands (region around 1600 cm^{-1} and 1400 cm^{-1}) from proteins, and the intense band around 1040 cm^{-1} from polysaccharides.

As can be seen in Fig. 4A, the composites HBx present bands common to the starting materials (hemp and bran) combined also with the absorption bands of the proteins from the self-grown mycelium (amide I at 1700 –1600 cm^{-1}, amide II and III at 1575 – 1300 cm^{-1}).

Table 2
Wettability results of mycelium-based materials.

	Sample Name	Contact Angle (°)
Hemp	HB0	123 ± 10
	HB10	126 ± 3
	HB20	131 ± 4
	HB30	124 ± 6
Cotton	CB0	121 ± 10
	CB10	132 ± 5
	CB20	138 ± 3
	CB30	114 ± 11

Table 3
Flexural properties of the mycelium-based materials.

	Sample Name	Modulus (MPa)	Strength at break (MPa)	Deformation at break (%)
Hemp	HB0	0.15 ± 0.06	0.04 ± 0.02	53.9 ± 29.2
	HB10	0.19 ± 0.02	0.10 ± 0.02	70.2 ± 15.5
	HB20	0.16 ± 0.01	0.07 ± 0.03	59.5 ± 26.7
	HB30	0.09 ± 0.01	0.03 ± 0.01	41.0 ± 7.8
Cotton	CB0	0.19 ± 0.03	0.05 ± 0.01	34.0 ± 2.3
	CB10	0.29 ± 0.08	0.07 ± 0.01	35.6 ± 9.8
	CB20	0.21 ± 0.06	0.08 ± 0.01	50.4 ± 10.0
	CB30	0.20 ± 0.04	0.06 ± 0.01	40.3 ± 9.2

ATR-FTIR spectra of the sample based on cotton are reported in Fig. 4B. The cotton fiber presents the strong signal observed at 3300 cm^{-1}, typical of the hydroxyl groups of cellulose, lignin and water. The absorption bands at 1414 and 1320 cm^{-1} are relative to bending vibrations of the C–H and C=O groups of the rings in cellulose. Intense signals observed at 1025 cm^{-1} are related to the (C=O) and (OH) stretching vibrations of the polysaccharide. Also in this case, cotton-based (CBx) material profiles show bands common to the starting materials (hemp, bran and mycelium), with the main absorbance around 3300 cm^{-1}, 2900 cm^{-1}, 1600 cm^{-1}, 1025 cm^{-1} corresponding mainly to cellulose/hemicellulose, waxes and oils, proteins and polysaccharides.

Therefore, both families of composites, HBx and CBx, feature bands common to the starting materials (hemp or cotton, bran and mycelium), thus proving that an interconnection exists between materials thanks to mycelium.

3.4. Static contact angle measurements

The static contact angle analysis (Table 2) was performed to assess the surface behavior of the mycelium-based composites and shows values around 120–130°. Such values agree with literature trends that report a hydrophobic behavior for the material's outer layer, entirely composed by pure mycelium.(Antinori et al., 2020) Since bran remains confined in the inner bulk, its influence on the surface properties is indirect, mainly affecting the development and the uniformity of the mycelium skin. Although associated to significantly high standard deviations, the presence of 10–20 % of bran seems to be linked to an increase of the hydrophobicity of the system HB20 and CB20 (Fig. 5). Such an effect might be due to the additional nutrient's contribution of bran for the mycelium growth, which stimulates the creation of a denser outer layer. A higher amount of bran (30 % wt), however, could destabilize the integrity of the inner bulk, by hampering the creation of a uniform peel, thus slightly decreasing the hydrophobicity of the material.

3.5. Mechanical behavior

The flexural behavior of all the samples was tested according to an adapted procedure reported as ISO 1209 and the obtained results are reported in Table 3 and displayed in Fig. 7.

As reported in Table 3, the presence of bran particles tends to increase the moduli, the strength and the extent of deformation of the material until a certain bran loading. Such a behavior could be explained by taking into consideration the different morphologies of hemp fibers and bran particles, and the final materials are the results of the properties of these two components. Hemp fibers impart high deformation while bran particles can reduce the voids in the composite, inducing higher strength and hardness. Moreover, although a significant standard deviation can be observed for the deformation at break values, a general trend can be envisaged and the sample containing 10 wt% (HB10) of bran seems the most performing one. In cotton-based materials (Table 3), the addition of bran is concomitant with a general improvement of the flexural properties, regardless of the amount used and the higher standard deviation in the deformation at break. In particular, CB20 is the best performing material, featuring high strength and high degree of deformation under flexural loads.

When comparing the two families of materials (Fig. 6), hemp-based samples proved to be less rigid with higher deformability even if it is reported that cotton fibers possess higher tensile elongation respect to hemp fibers.(Latif et al., 2019) However, cotton-based samples feature a good compromise between stiffness and deformability, in particular the sample containing 20 wt% of wheat bran.

The evaluation of the compressive performances of the synthesized materials were performed according to an adapted procedure of ISO 844 and the results are reported in Table 4 and Fig. 7.

The compression strength was obtained from the stress-strain relationships for each sample at fixed strain because no peaks occurred in the stress-strain relationship (strain-hardening behavior). As shown in Table 4, all the samples based on hemp fibers and containing bran particles perform better with respect to the reference material (HB0). In the case of the cotton series, however, bran only marginally affected the compression strength. Such a behavior is explained by the tendency of bran to reduce the voids in the composite, thus increasing the rigidity, the strength and the extent of deformation of the material inducing higher strength and stiffness. As a matter of fact, cotton fibers manage to obtain more compact mats presenting a lower ratio of void space, thus having a limited benefit from bran's contribution.

The mechanical performances of the previously described materials can be easily benchmarked within the family of mycelium-based materials. Apparently, the flexural strength in mycelium-based materials can vary in a wide range according to the different substrates involved in the fungal growth. Holt et al.,(Holt et al., 2012) for example, report values in between 0.007 MPa and 0.26 MPa, while Yang et al.(Yang et al., 2017) have values ranging from 0.0046 MPa and 0.18 MPa. Higher values were found by Appels et al.(Appels et al., 2019) obtaining mycelium-based

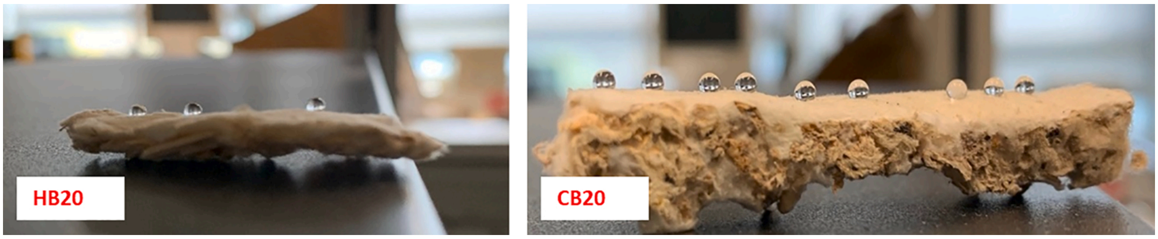


Fig. 5. water drops on the outer layer of samples HB20 and CB20.

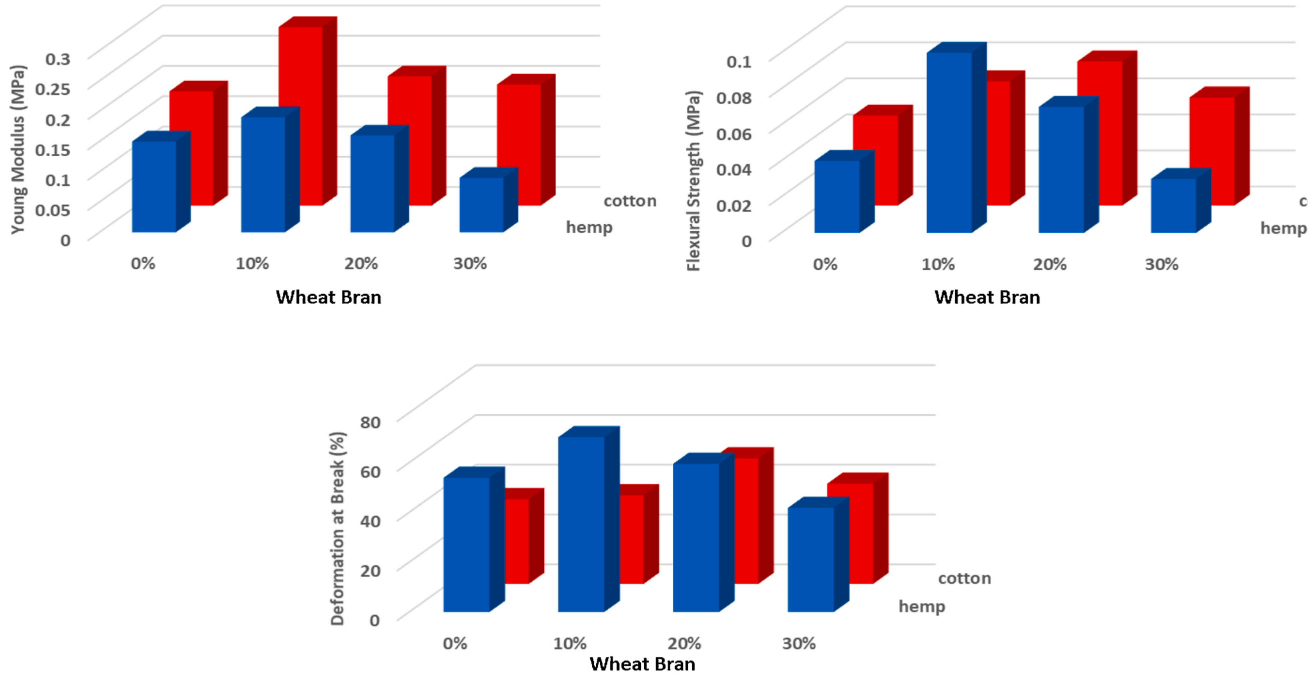


Fig. 6. Flexural modulus, strength and deformation at break of the mycelium-based materials.

materials with flexural strengths varying from 0.05 to 0.29 MPa and flexural moduli from 1 to 9 MPa. Concerning the compressing strength, mycelium based foams range between 0.029 MPa and 0.567 MPa. (Girometta et al., 2019) Within the framework of the classical packaging materials, the hereby reported bran/mycelium composites can compete with expanded polystyrene (EPS). EPS flexural strength, as well as its compressive strength, range between 0.07 and 0.69 MPa.(López Nava et al., 2016)

3.6. *Compostability test*

An overview of the disintegration in simulated industrial composting conditions of the materials obtained using cotton/bran and hemp/bran mixtures is given in Fig. 8 and 9, respectively. The cotton based materials were tested for a thickness varying between 26 mm and 29 mm, while the hemp materials HB0 and HB10 showed a higher thickness of 40 mm and 45 mm, respectively. The materials with higher bran content

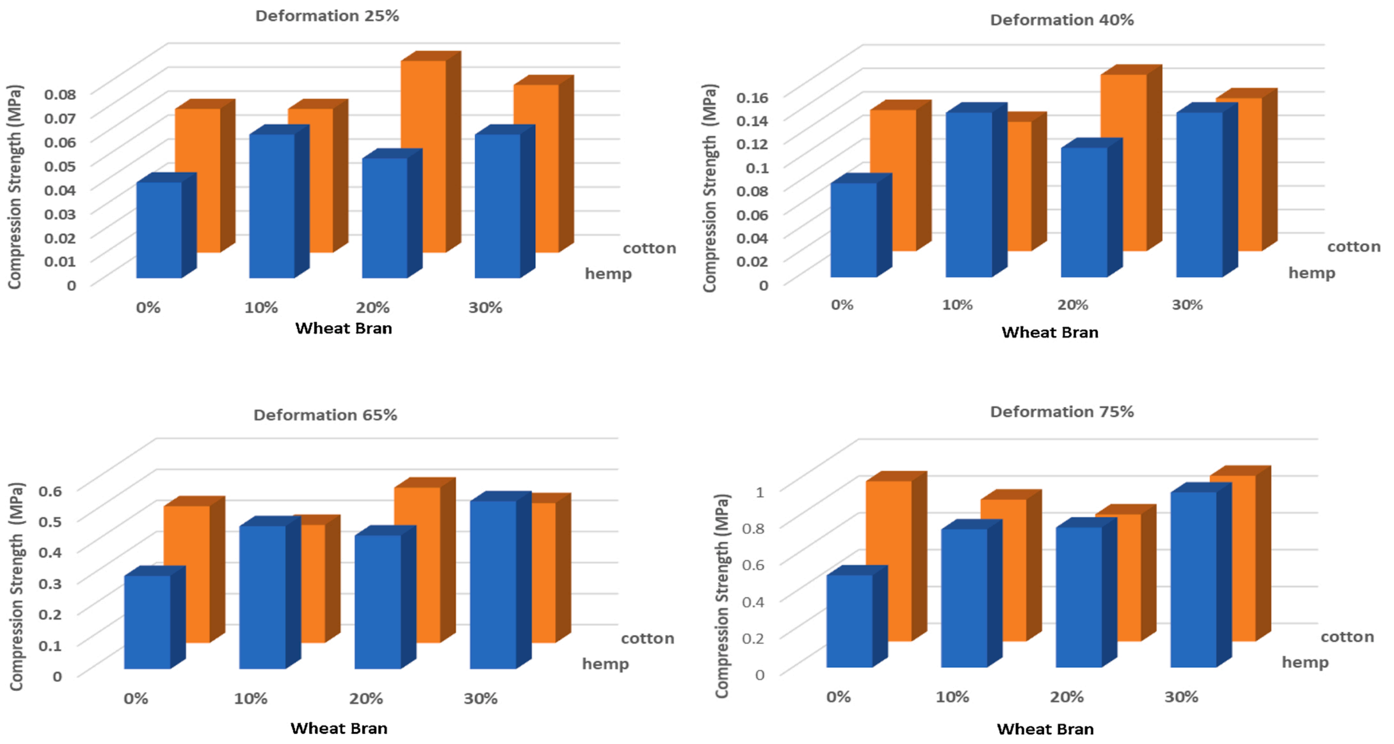


Fig. 7. Compression strength of the mycelium-based materials at 25 %, 40 %, 65 % and 75 % of deformation.

Table 4
Compression properties of the composites.

	Sample Name	Strength at 25 % deformation (MPa)	Strength at 40 % deformation (MPa)	Strength at 65 % deformation (MPa)	Strength at 75 % deformation (MPa)
Hemp	HB0	0.04 ± 0.01	0.08 ± 0.01	0.30 ± 0.01	0.53 ± 0.01
	HB10	0.06 ± 0.01	0.14 ± 0.02	0.46 ± 0.06	0.82 ± 0.11
	HB20	0.05 ± 0.01	0.11 ± 0.02	0.43 ± 0.09	0.79 ± 0.18
	HB30	0.06 ± 0.01	0.14 ± 0.02	0.54 ± 0.05	1.00 ± 0.06
Cotton	CB0	0.06 ± 0.01	0.12 ± 0.02	0.44 ± 0.07	0.87 ± 0.17
	CB10	0.06 ± 0.01	0.11 ± 0.01	0.38 ± 0.02	0.77 ± 0.05
	CB20	0.08 ± 0.01	0.15 ± 0.01	0.50 ± 0.02	0.69 ± 0.51
	CB30	0.07 ± 0.01	0.13 ± 0.02	0.45 ± 0.04	0.90 ± 0.08

HB20 and HB30 had a thickness of 29 mm and 32 mm. Thickness can play an important role in the disintegration rate. At the start of the composting process the test materials were added as cubes of about 5 cm × 5 cm and mixed with biowaste. The biowaste consisted of a mixture of fresh Vegetable, Garden and Fruit waste (VGF) and structural material. The obtained mixture was aerobically composted for the prescribed duration of 12 weeks in 200 L composting bins. After start-up the temperature increased almost immediately up to over 60 °C. The temperature profile showed an initial thermophilic phase and a mesophilic continuation, which is representative of industrial composting. Moreover, the oxygen concentration of the exhaust air remained above 10 % during the test, which guaranteed good aeration conditions. The disintegration of the cotton series proceeded slowly. At the end of the test, still large test item pieces were retrieved. The disintegration was clearly insufficient. For material CB0 (28 mm) the cubes had fallen apart into pieces, while for the cotton/bran mixtures the pieces were still rather intact. This is most likely linked with the denser mycelium network and extensive aggregates when adding bran. However, as the materials were tested in quite high thicknesses, it's still possible that compostability can be obtained for lower thicknesses. The disintegration of the hemp/bran mixtures proceeded better compared to the cotton mixtures. After 10 weeks of composting HB0 (40 mm) and HB20 (29 mm) had completely disappeared. This was confirmed during a thorough examination at the end of the test. Such materials are compostable under industrial composting conditions. Again the effect of bran was observed. While HB0 without bran with a 40 mm thickness was completely disintegrated, still big pieces were retrieved for HB10 (10 % bran) in a similar thickness of 43 mm. HB20 with a bran content of 20 % showed complete disintegration, but this material was tested in a significantly lower thickness. By reducing the thickness, complete disintegration under industrial composting conditions might also be obtained for the other grades. As lignocellulosic materials tend to degrade better under home composting conditions with lower temperatures and increased activity of fungi and actinomycetes (Singer et al., 2000) when compared to industrial conditions, the materials are also potentially home-compostable.

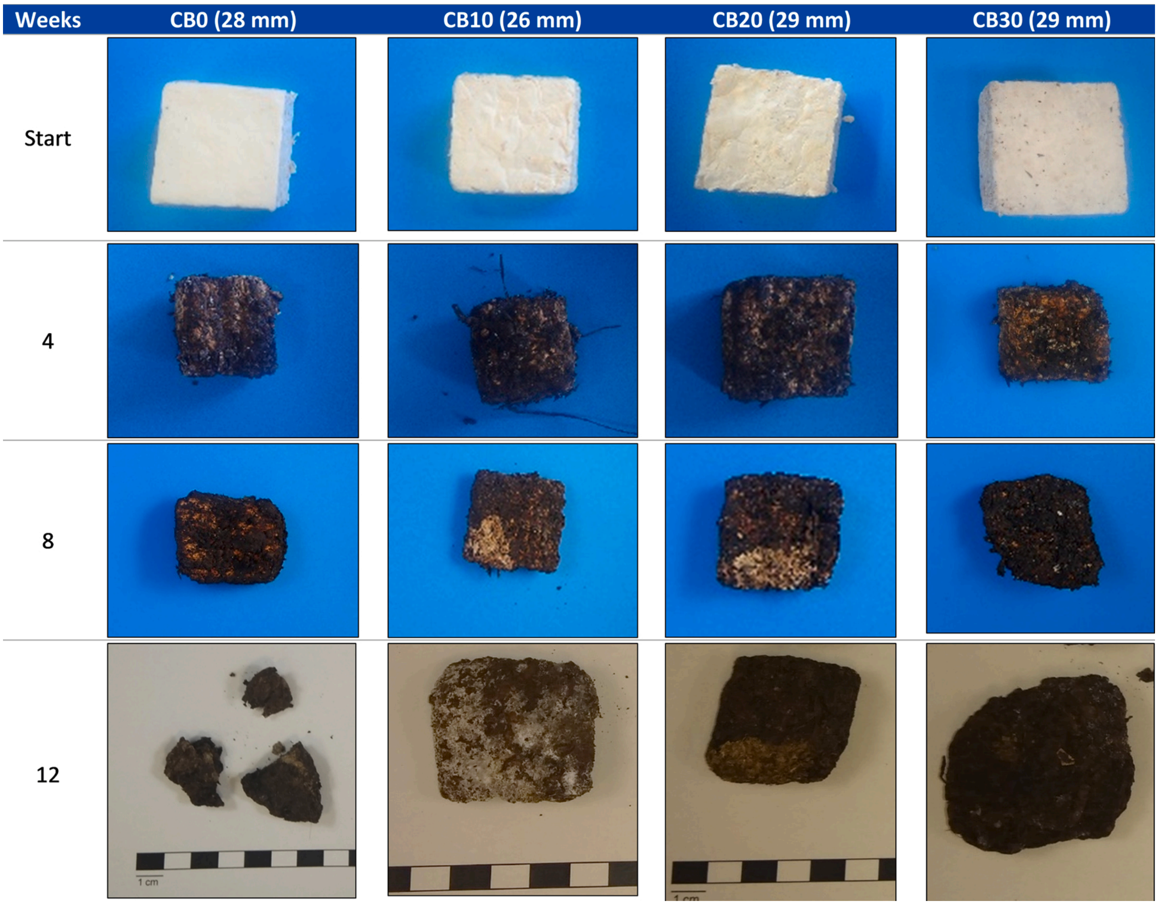


Fig. 8. Visual representation of the disintegration of cotton/bran mycelium-based materials during a 12 weeks pilot-scale composting test.

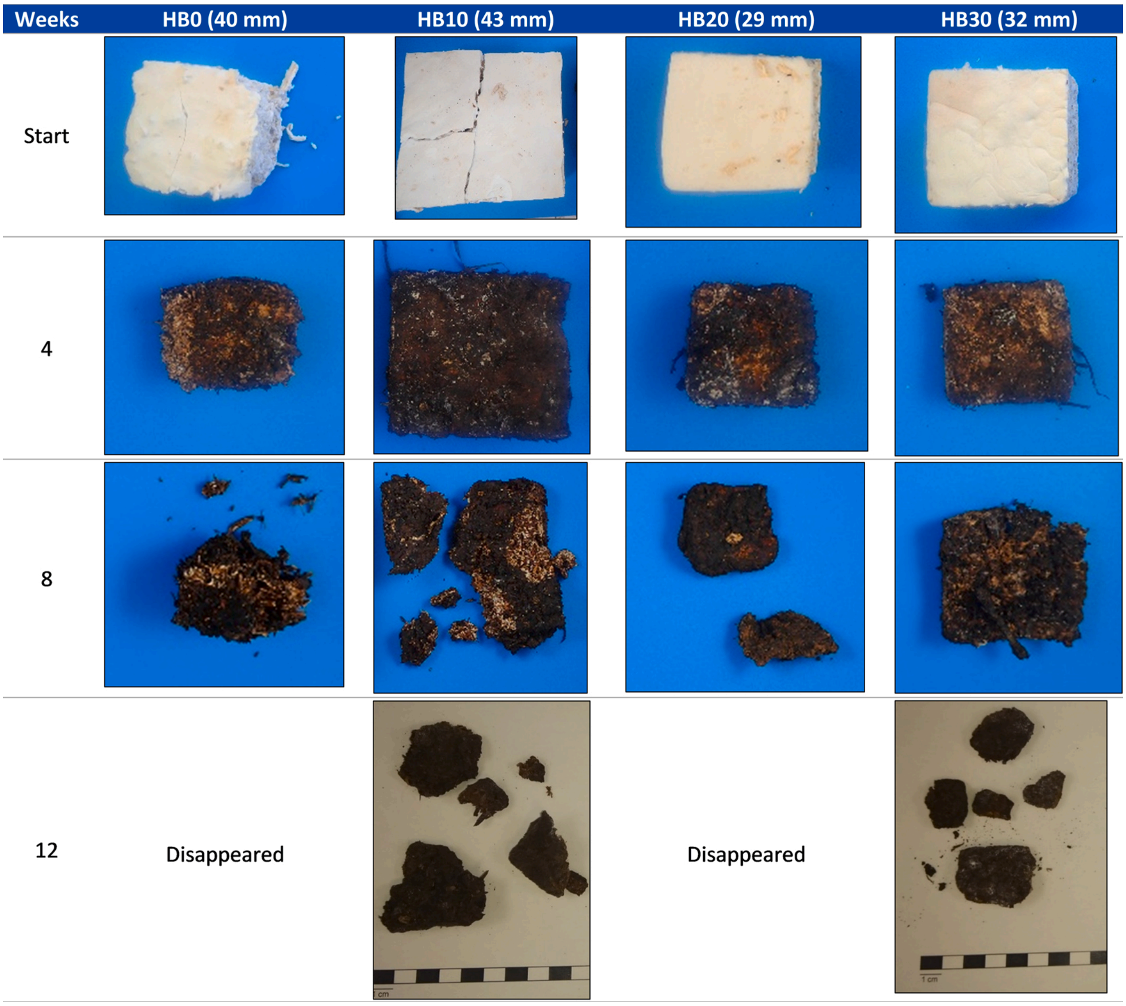


Fig. 9. Visual representation of the disintegration of hemp/bran mycelium-based materials during a 12 weeks pilot-scale composting test.

4. Conclusions

The effect of industrial residues of wheat bran as a supplement and reinforcing agent for the fabrication of mycelium-based materials at an industrial level was systematically studied. As a feedstock for fungal development, bran proved to confer a pool of desirable nutrients capable to boost the mycelium growth speed as well as to favor a more homogeneous, thick and hydrophobic surface. Such aspects provide outstanding advantages from an industrial perspective since productivity is heavily dependent on the fungal growth stage while the creation of a flourishing outer layer allows to design stable products, poorly affected by humid environments. In addition, bran can modify the mechanical behavior of the mycelium composite by acting as reinforcing agent. While hemp and cotton fibers impart high deformation, bran particles synergistically increase strength and hardness by reducing the voids in the material. As a consequence, an increasing of the flexural modulus, flexural strength and deformation at break was observed for relatively low amount of bran. Compression properties also benefitted from the voids reduction offered by bran, specifically for hemp-based composites. The possibility to increase and control the mechanical properties of these materials allows to challenge a classical petrol-based packaging material such as expanded polystyrene for the creation of novel market sectors. Finally, the mycelium-based materials showed an unexpected resilience in composting conditions reaching complete disintegration only in limited occasions. The reason for such a behavior can be found in the high lignocellulosic content of such materials, that confers additional stability to the artifacts which can find their end-life as compost in domestic composters. As a conclusion, within the framework of the currently vibrant bio-economy scenario and inspired by the principles of biorefinery, we demonstrated that the valorization of the industrial residue of wheat bran open new possibilities of developing fully sustainable materials.

Funding

This work was supported by AGRIMAX and funded by the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 Research and Innovation Program under grant agreement No. 720719.

CRediT authorship contribution statement

Laura Sisti: Conceptualization, Investigation, Data curation, Supervision. **Claudio Gioia:** Conceptualization, Investigation, Data curation, Writing - original draft. **Grazia Totaro:** Investigation, Data curation. **Steven Verstichel:** Investigation, Data curation. **Marco Cartabia:** Investigation, Data curation. **Serena Camere:** Supervision. **Annamaria Celli:** Funding acquisition, Supervision.

Declaration of Competing Interest

Annamaria Celli reports financial support was provided by Bio Based Industries Joint Undertaking under the European Union. Marco Cartabia, Serena Camere reports a relationship with MOGU that includes: employment. Steven Verstichel reports a relationship with OWS that includes: employment.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2021.113742.

References

Abhijith, R., Ashok, A., Rejeesh, C.R., 2018. Sustainable packaging applications from mycelium to substitute polystyrene: a review. Mater. Today Proc. 5, 2139–2145. https://doi.org/10.1016/j.matpr.2017.09.211.

https://agromax.iris.cat/, n.d.

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

Appels, F.V.W., Camere, S., Montalti, M., Karana, E., Jansen, K.M.B., Dijksterhuis, J., Krijgsheld, P., Wösten, H.A.B., 2019. Fabrication factors influencing mechanical, moisture- and water-related properties of mycelium-based composites. Mater. Des. 161, 64–71. https://doi.org/10.1016/j.matdes.2018.11.027.

Apprich, S., Tirpanalan, Ö., Hell, J., Reisinger, M., Böhmdorfer, S., Siebenhandl-ehn, S., Novalin, S., Kneifel, W., 2014. Wheat bran-based biore finery 2 : valorization of products. LWT - Food Sci. Technol. 56, 222–231. https://doi.org/10.1016/j.lwt.2013.12.003.

Chandrasekaran, M., 2012. Valorization of food processing by-products. Valorization of Food Processing By-Products. https://doi.org/10.1201/b12816.

D'Amato, D., Droste, N., Allen, B., Kettunen, M., Lähtinen, K., Korhonen, J., Leskinen, P., Matthies, B.D., Toppinen, A., 2017. Green, circular, bio economy: a comparative analysis of sustainability avenues. J. Clean. Prod. 168, 716–734. https://doi.org/10.1016/j.jclepro.2017.09.053.

Dahmen, N., Henrich, E., Henrich, T., 2019. Synthesis gas biorefinery. Adv. Biochem. Eng. Biotechnol. https://doi.org/10.1007/10_2016_63.

Elmekawy, A., Diels, L., De Wever, H., Pant, D., 2013. Valorization of cereal based biorefinery byproducts: reality and expectations. Environ. Sci. Technol. 47, 9014–9027. https://doi.org/10.1021/es402395g.

Elsacker, E., Vandelook, S., Brancart, J., Peeters, E., De Laet, L., 2019. Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates. PLoS One 14, 1–20. https://doi.org/10.1371/journal.pone.0213954.

Formela, K., Hejna, A., Piszczyk, Ł., Saeb, M.R., Colom, X., 2016. Processing and structure–property relationships of natural rubber/wheat bran biocomposites. Cellulose 23, 3157–3175. https://doi.org/10.1007/s10570-016-1020-0.

Fritsch, C., Staebler, A., Happel, A., Márquez, M.A.C., Aguiló-Aguayo, I., Abadias, M., Gallur, M., Cigognini, I.M., Montanari, A., López, M.J., Suárez-Estrella, F., Brunton, N., Luengo, E., Sisti, L., Ferri, M., Belotti, G., 2017. Processing, valorization and application of bio-waste derived compounds from potato, tomato, olive and cereals: a review. Sustain 9, 1–46. https://doi.org/10.3390/su9081492.

Germec, M., Ozcan, A., Turhan, I., 2019. Bioconversion of wheat bran into high value-added products and modelling of fermentations. Ind. Crops Prod. 139, 111565 https://doi.org/10.1016/j.indcrop.2019.111565.

Ghisellini, P., Cialani, C., Ulgiati, S., 2016. A review on circular economy: the expected transition to a balanced interplay of environmental and economic systems. J. Clean. Prod. 114, 11–32. https://doi.org/10.1016/j.jclepro.2015.09.007.

Gigante, V., Cinelli, P., Righetti, M.C., Sandroni, M., Polacco, G., Seggiani, M., Lazzeri, A., 2020. On the use of biobased waxes to tune thermal and mechanical properties of polyhydroxyalkanoates– bran biocomposites. Polymers (Basel). 12, 1–18. https://doi.org/10.3390/polym12112615.

Girometta, C., Picco, A.M., Baiguera, R.M., Dondi, D., Babbini, S., Cartabia, M., Pellegrini, M., Savino, E., 2019. Physico-mechanical and thermodynamic properties of mycelium-based biocomposites: a review. Sustain 11. https://doi.org/10.3390/su11010281.

Haneef, M., Ceseracciu, L., Canale, C., Bayer, I.S., Heredia-Guerrero, J.A., Athanassiou, A., 2017. Advanced materials from fungal mycelium: fabrication and tuning of physical properties. Sci. Rep. 7, 1–11. https://doi.org/10.1038/srep41292.

Hejna, A., Barczewski, M., Andrzejewski, J., Kosmela, P., Piasecki, A., Szostak, M., Kuang, T., 2020. Rotational molding of linear low-density polyethylene composites filled with wheat bran. Polymers (Basel). 12, 1004. https://doi.org/10.3390/POLYM12051004.

Holt, G.A., McIntyre, G., Flagg, D., Bayer, E., Wanjura, J.D., Pelletier, M.G., 2012. 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. Bioenergy 6, 431–439. https://doi.org/10.1166/jbmb.2012.1241.

Islam, M.R., Tudryn, G., Bucinell, R., Schadler, L., Picu, R.C., 2017. Morphology and mechanics of fungal mycelium. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-13295-2.

Jones, M., Huynh, T., Dekiwadia, C., Daver, F., John, S., 2017. Mycelium composites: a review of engineering characteristics and growth kinetics. J. Bionanoscience 11, 241–257. https://doi.org/10.1166/jbns.2017.1440.

Jones, M., Bhat, T., Huynh, T., Kandare, E., Yuen, R., Wang, C.H., John, S., 2018a. Waste-derived low-cost mycelium composite construction materials with improved fire safety. Fire Mater. 42, 816–825. https://doi.org/10.1002/fam.2637.

Jones, M., Bhat, T., Kandare, E., Thomas, A., Joseph, P., Dekiwadia, C., Yuen, R., John, S., Ma, J., Wang, C.H., 2018b. Thermal Degradation and Fire Properties of Fungal Mycelium and Mycelium - Biomass Composite Materials. Sci. Rep. 8, 1–10. https://doi.org/10.1038/s41598-018-36032-9.

Jones, M., Weiland, K., Kujundzic, M., Theiner, J., Kählig, H., Kontturi, E., John, S., Bismarck, A., Mautner, A., 2019a. Waste-derived low-cost mycelium nanopapers with tunable mechanical and surface properties. Biomacromolecules 20, 3513–3523. https://doi.org/10.1021/acs.biomac.9b00791.

Jones, M.P., Lawrie, A.C., Huynh, T.T., Morrison, P.D., Mautner, A., Bismarck, A., John, S., 2019b. Agricultural by-product suitability for the production of chitinous composites and nanofibers utilising Trametes versicolor and Polyporus brumalis mycelial growth. Process Biochem. 80, 95–102. https://doi.org/10.1016/j.procbio.2019.01.018.

Joshi, K., Meher, M.K., Poluri, K.M., 2020. Fabrication and characterization of bioblocks from agricultural waste using fungal mycelium for renewable and sustainable applications. ACS Appl. Bio Mater. 3, 1884–1892. https://doi.org/10.1021/acsabm.9b01047.

Kavanagh, K., 2005. Fungi : Biology and Applications, 3rd edition.

Latif, R., Wakeel, S., Khan, N.Z., Noor Siddiquee, A., Lal Verma, S., Akhtar Khan, Z., 2019. Surface treatments of plant fibers and their effects on mechanical properties of fiber-reinforced composites: a review. J. Reinf. Plast. Compos. 38, 15–30. https://doi.org/10.1177/0731684418802022.

Leung, P.H., Zhang, Q.X., Wu, J.Y., 2006. Mycelium cultivation, chemical composition and antitumour activity of a Tolypocladium sp. Fungus isolated from wild Cordyceps sinensis. J. Appl. Microbiol. 101, 275–283. https://doi.org/10.1111/j.1365-2672.2006.02930.x.

Liu, Y., Kim, H.J., 2017. Fourier transform infrared spectroscopy (FT-IR) and simple algorithm analysis for rapid and non-destructive assessment of developmental cotton fibers. Sensors (Switzerland) 17. https://doi.org/10.3390/s17071469.

Liu, R., Long, L., Sheng, Y., Xu, J., Qiu, H., Li, X., Wang, Y., Wu, H., 2019. Preparation of a kind of novel sustainable mycelium/cotton stalk composites and effects of pressing temperature on the properties. Ind. Crops Prod. 141, 111732 https://doi.org/10.1016/j.indcrop.2019.111732.

Londo, M., van Stralen, J., Uslu, A., Mozaffarian, H., Kraan, C., 2018. Lignocellulosic biomass for chemicals and energy: an integrated assessment of future EU market sizes, feedstock availability impacts, synergy and competition effects, and path dependencies. Biofuels, Bioprod. Biorefining 12, 1065–1081. https://doi.org/10.1002/bbb.1926.

López Nava, J.A., Méndez González, J., Ruelas Chacón, X., Nájera Luna, J.A., 2016. Assessment of edible Fungi and films bio-based material simulating expanded polystyrene. Mater. Manuf. Process. 31, 1085–1090. https://doi.org/10.1080/10426914.2015.1070420.

Nawawi, W.M.F.W., Jones, M.P., Kontturi, E., Mautner, A., Bismarck, A., 2020. Plastic to elastic: fungi-derived composite nanopapers with tunable tensile properties. Compos. Sci. Technol. 198 https://doi.org/10.1016/j.compscitech.2020.108327.

Onipe, O.O., Jideani, A.I.O., Beswa, D., 2015. Composition and functionality of wheat bran and its application in some cereal food products. Int. J. Food Sci. Technol. 50, 2509–2518. https://doi.org/10.1111/ijfs.12935.

Pan, Z., Cathcart, A., Wang, D., 2006. Properties of particleboard bond with rice bran and polymeric methylene diphenyl diisocyanate adhesives. Ind. Crops Prod. 23, 40–45. https://doi.org/10.1016/j.indcrop.2005.03.004.

Pelletier, M.G., Holt, G.A., Wanjura, J.D., Lara, A.J., Tapia-Carillo, A., McIntyre, G., Bayer, E., 2013. An evaluation study of mycelium based acoustic absorbers grown onagricultural by-product substrates. Ind. Crops Prod. 51, 480–485. https://doi.org/10.1016/j.indcrop.2016.10.042.

Pinales-Márquez, C.D., Rodríguez-Jasso, R.M., Araújo, R.G., Loredo-Treviño, A., Nabarlatz, D., Gullón, B., Ruiz, H.A., 2021. Circular bioeconomy and integrated biorefinery in the production of xylooligosaccharides from lignocellulosic biomass: a review. Ind. Crops Prod. 162 https://doi.org/10.1016/j.indcrop.2021.113274.

Prospects, C., Situation, F., 2020. Crop Prospects and Food Situation #4, December 2020. Crop Prospects and Food Situation #4. https://doi.org/10.4060/cb2334en. December 2020.

Rahman, A., Ulven, C., Durant, C., Johnson, M., Fehrenbach, J., Hossain, K.G., 2017. Selection, Pretreatment, and Use of Wheat Bran for Making Thermoplastic Composite. 2017 ASABE Annu. Int. Meet. https://doi.org/10.13031/aim.201701090

Rudjito, R.C., Ruthes, A.C., Jiménez-Quero, A., Vilaplana, F., 2019. Feruloylated arabinoxylans from wheat bran: optimization of extraction process and validation at pilot scale. ACS Sustain. Chem. Eng. 7, 13167–13177. https://doi.org/10.1021/acssuschemeng.9b02329.

Ruthes, A.C., Rudjito, R.C., Rencoret, J., Gutiérrez, A., del Río, J.C., Jiménez-Quero, A., Vilaplana, F., 2020. Comparative recalcitrance and extractability of cell wall polysaccharides from cereal (Wheat, rye, and barley) brans using subcritical water. ACS Sustain. Chem. Eng. 8, 7192–7204. https://doi.org/10.1021/acssuschemeng.0c01764.

Shankar, R.S., Srinivasan, S.A., Shankar, S., Rajasekar, R., Kumar, R.N., Kumar, P.S., 2014. Review article on wheat flour/wheat bran/wheat husk based bio composites. Int. J. Sci. Res. Publ. 4, 1–9.

Sherwood, J., 2020. The significance of biomass in a circular economy. Bioresour. Technol. 300 https://doi.org/10.1016/j.biortech.2020.122755.

Singer, A., Crohn, D., Humpert, C.P., 2000. Compost microbiology and the soil food web. Calif. Integr. Waste Manag. Board 442, 0–13.

Sisti, L., Totaro, G., Vannini, M., Fabbri, P., Kalia, S., Zatta, A., Celli, A., 2016. Evaluation of the retting process as a pre-treatment of vegetable fibers for the preparation of high-performance polymer biocomposites. Ind. Crops Prod. 81, 56–65. https://doi.org/10.1016/j.indcrop.2015.11.045.

Sohn, J.S., Kim, H.K., Cha, S.W., 2019. Bio-based foamed cushioning materials using polypropylene and wheat bran. Sustain 11, 1670. https://doi.org/10.3390/su11061670.

Sun, W., Tajvidi, M., Hunt, C.G., McIntyre, G., Gardner, D.J., 2019. Fully bio-based hybrid composites made of wood, fungal mycelium and cellulose nanofibrils. Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-40442-8.

Sun, W., Tajvidi, M., Hunt, C.G., Howell, C., 2021. All-natural smart mycelium surface with tunable wettability. ACS Appl. Bio Mater 4, 1015–1022. https://doi.org/10.1021/acsabm.0c01449.

Ubando, A.T., Felix, C.B., Chen, W.H., 2020. Biorefineries in circular bioeconomy: a comprehensive review. Bioresour. Technol. 299 https://doi.org/10.1016/j.biortech.2019.122585.

Wang, J., Yuan, X., Sun, B., Cao, Y., Tian, Y., Wang, C., 2009. On-line separation and structural characterisation of feruloylated oligosaccharides from wheat bran using HPLC-ESI-MSn. Food Chem. 115, 1529–1541. https://doi.org/10.1016/j.foodchem.2009.01.058.

Yang, Z.(Joey), Zhang, F., Still, B., White, M., Amstislavski, P., 2017. Physical and mechanical properties of fungal mycelium-based biofoam. J. Mater. Civ. Eng. 29, 04017030 https://doi.org/10.1061/(asce)mt.1943-5533.0001866.

Zhang, Y., Ge, X., Deng, F., Li, M.C., Cho, U.R., 2017. Fabrication and characterization of rice bran carbon/styrene butadiene rubber composites fabricated by latex compounding method. Polym. Compos. 38, 2594–2602. https://doi.org/10.1002/pc.23852.