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Research article

Physical and Mechanical Properties of Indian Oyster Mushroom Mycelium/Sawdust Composites for Biodegradable Packaging Materials

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Abstract

Mycelium-based composite (MBC) offers an excellent sustainable alternative to hydrocarbon-based materials, especially styrofoam for packaging, due to its abundance of fungal mycelium that grows quickly on agricultural substrates, its biodegradable and its lightweight. The mycelium of a commercial mushroom species, *Pleurotus ostreatus* (PO), is used to fabricate MBC for packaging materials. Another species, *Pleurotus pulmonarius* (PP), prefers warmer weather, making it more common in tropical countries. Nevertheless, there is a lack of studies of PP mycelium-based composites and their mechanical and physical properties. This study investigated the physical and mechanical properties of PP mycelium/sawdust composite and compared to PO mycelium/sawdust composite. The results showed that the average density of PP/sawdust and PO/sawdust composites were 292.14 and 272.17 kg/m^3, respectively, which fell within the range of low-density polyurethane foam. The final mass gain due to water absorption into PO/sawdust specimens was 144.04%, 1.41 times lower than PP/sawdust specimens. Furthermore, PP/sawdust composite exhibited 7.5 times faster water absorption rate than PO/sawdust composite, indicating that PO/sawdust had better water resistance. The PP/sawdust composite produced an equivalent compressive modulus to the PO/sawdust composite under compression up to 1.34 MPa of maximum value. Thus, the PP/sawdust composite showed excellent potential for substitution of biodegradable packages made from PO/sawdust composite as they contributed the equivalent strength; however, the PO/sawdust composite exhibited superior water resistance to the PP/sawdust composite. Consequently, PO/sawdust should be more advantageous if the biodegradable packaging is required to be of strength as high as the low-density polyurethane foam and of compatible water resistance.

Keywords: mycelium-based composite; biodegradable packaging; *Pleurotus pulmonarius*; *Pleurotus ostreatus*; mechanical properties; physical properties; water absorption; sustainable materials

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1. Introduction

The need to reduce the use of petroleum resources and minimizing their waste has become a serious issue recently. To achieve the goal of zero waste, bio-based materials derived from natural resources as well as recycled waste products, for example, eggshell (Bootklad & Kaewtatip, 2013; Chong et al., 2020), seashell (Owuamanam & Cree, 2020), rice husk (Sun & Gong, 2001; Thomas, 2018), and natural fiber-based composites (Gholampour & Ozbakkaloglu, 2020; Rashid et al., 2020) are alternative options that can replace hydrocarbon-based materials as they are biodegradable and eco-friendly. Nowadays, mycelium-based composites (MBCs) are attractive among bio-based materials since mushroom is a fungus of the largest living organism on Earth. Mycelial growth of mushrooms naturally forms a three-dimensional network of filamentous fungi on agricultural substrates. Therefore, MBCs are biodegradable, lightweight and produce low levels of carbon emissions during manufacturing. Most of the works reported that MBCs exhibited advantageous properties such as fire-retardant (Chulikavit et al., 2023) and insulation (Zhang et al. 2022; Zhang et al. 2023) with the strength and density for utilization as biodegradable packaging materials (Holt et al., 2012).

Studies of MBCs started with the selection of fungi, substrates for mycelial growth, and a suitable growth environment for selected fungi species. The effects of types and sizes of substrates on the mechanical properties of MBC and growth of mycelium on various substrates have been reported in numerous studies. Elsacker et al. (2018) reported that mycelium grew well on the lignocellulosic substrates, flax and hemp, and the small particle size of substrates enhanced the compressive stiffness of the MBC. The most frequently cited families for fabrication of MBC are *Trametes* spp., *Ganoderma* spp., and *Pleurotus* spp. *Trametes versicolor*, commonly known as ‘Turkey tail mushroom’, can be easily found in forests as they usually grow by breaking down the decaying hornbeam wood. *Ganoderma lucidum*, commonly known as ‘Lingzhi’, contains versatile ligninolytic enzymes: laccase, lignin peroxidase, manganese peroxidase, which can degrade lignocellulosic waste and this break down of lignin supports growth (Soh et al., 2021; Rigobello & Ayres, 2022). *Pleurotus ostreatus*, commonly known as ‘Oyster mushroom’, can also grow well by degradation of solid lignocellulosic substrates. The fruiting bodies of *G. lucidum* and *P. ostreatus* are widely used since they contain medicinal properties and are edible. Therefore, *G. lucidum* and *P. ostreatus* can be found more easily in local supermarket than *T. versicolor*. Several reports disclosed that the mycelial growth rate of *G. lucidum* and *T. versicolor* is 7-9 days, while the mycelium of *P. ostreatus* fully grows on lignocellulosic substrates within 22-28 days (Jones et al., 2019; Soh et al., 2021; Chulikavit et al., 2022; Rigobello & Ayres, 2022). Moreover, suitable conditions for the mycelial growth of *T. versicolor*, *G. lucidum*, and *P. ostreatus* on lignocellulosic substrates are similar at 22-28°C, and 65-80% RH (Fletcher et al., 2019; Koutrotsios et al., 2019; Sydor et al., 2022). It was reported that the MBC fabricated from the mycelium of *P. ostreatus* grown on cellulose substrate was stiffer than that of *G. lucidum* (Haneef et al., 2017). Among these three types of fungi, *P. ostreatus* is the cheapest and is usually found in local supermarkets. Nevertheless, the other species of *Pleurotus* spp. have not been thoroughly studied for the fabrication, physical and mechanical properties of the MBC.

Pleurotus pulmonarius, commonly known as ‘Indian oyster mushroom’, prefers warmer weather to *P. ostreatus*. It is mostly found in China, Thailand, New Zealand and appears in warmer seasons in North Europe. Therefore, it is the most popular oyster mushroom species and abundantly available throughout the year in tropical countries of Southeast Asia. The appearance of *P. ostreatus* and *P. pulmonarius* is almost similar and both are commercial mushroom species. The cap of *P. ostreatus* is rounder than that of oysters and its cap can be either white or gray. However, the cap of *P. pulmonarius* is smaller than *P. ostreatus* cap which is paler in color and lung-shaped and its stem is slightly larger than *P. ostreatus* (Yu et al., 2024).

To the best of the authors' knowledge, there are few literatures regarding the utilization of *P. pulmonarius* mycelium-based lignocellulosic composites. Khorsheed & Ahmed (2023) investigated the feasibility of using locally-available substrates with supplements from agricultural waste to cultivate *P. pulmonarius*. Their work was focused on the appearance of fruiting bodies, not on the fabrication of MBC. Attias et al. (2017) studied the growth characteristics of the mycelia of *P. pulmonarius, P. ostreatus, P. salmoneo* and *Aaegerita agrocibe* grown on five different lignocellulosic substrates. They found that *P. pulmonarius* and *P. ostreatus* grew better on apple and vine woodchips rather than *P. salmoneo* and *A. agrocibe*. Additionally, they found that the humidity in the specimens did not correlate with mycelium density. Based on these published works, the mycelium of *P. pulmonarius* could be used in MBC fabrication as its fungal growth speed, availability, price, and growth conditions were comparable to those of *P. ostreatus*. Nonetheless, information on the mechanical and physical properties of MBC produced from the mycelium of *P. pulmonarius* was not mentioned in any published work.

This study aimed to investigate the physical and mechanical properties of fungal mycelia of *P. pulmonarius* and *P. ostreatus* grown on sawdust particles. The mushroom spawn of both fungi was received from a certified commercial mushroom farm in Lamphun province, Thailand, and molded into the desired shape. The dry density and water absorption rates were investigated. Compression tests were carried out. The compressive modulus of the specimens was determined from the slope of the stress-strain curve obtained from the compression test and the results were compared with the literature. The outside skin and morphology of the mycelia were observed using a digital microscope. The results of this work were anticipated to understand the physical and mechanical properties as well as the mycelium morphology of *P. pulmonarius* and its suitability for low-cost biodegradable packaging material applications.

2. Materials and Methods

2.1 Fabrication process and conditions

Figure 1 illustrates the fabrication conditions and process of *P. pulmonarius* (hereafter referred to as "PP") and *P. ostreatus* (hereafter referred to as "PO") mushrooms mycelium/sawdust composites. The mushroom spawns mixed with the sterilized para-rubber wood sawdust packages were purchased from a certified commercial mushroom farm (Thai Agricultural Standard 2501-2559) in Lamphun province, Thailand (the mushroom spawns in the sawdust package are typically sent out from the farm to the customers after the mycelium has grown and covered half of the package). The averaged initial weight of the sawdust with mushroom spawn packages was 875 g. The packages were inoculated until mycelium had covered all the sawdust particles to ensure that the mycelium had homogeneously formed a 3D filamentous network with the sawdust particles before the package was to be broken and molded into the desired shape. According to the literature, the dominant factors affecting oyster mushroom mycelium growth arranged in decreasing order of significant factors: (i) temperature, (ii) relative humidity, (iii) type of substrate, (iv) light, and (v) oxygen (Zharare et al., 2010; Anukwuorji et al., 2023;

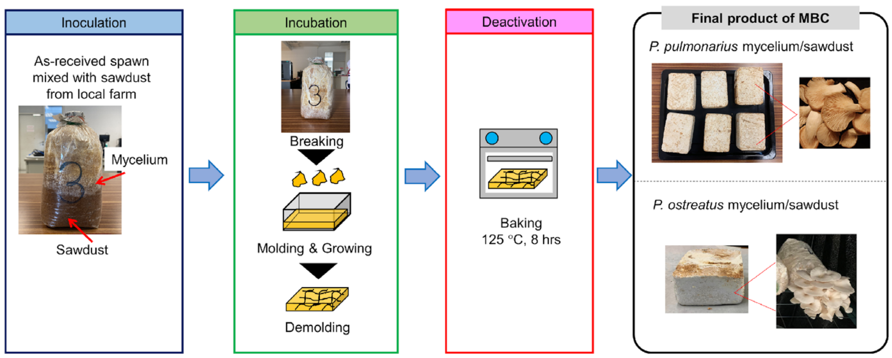

Figure 1. Fabrication conditions and process of *P. pulmonarius* mycelium/sawdust composites and *P. ostreatus* mycelium/sawdust composites.

Rungjindamai et al., 2024). Mycelial growth of *P. ostreatus* prefers 25-30ºC temperatures, while *P. pulmonarius* prefer warmer temperatures of 28-30ºC. The suitable temperatures for the growth of the fruiting bodies of *P. ostreatus* and *P. pulmonarius* are 25ºC and 28ºC, respectively. The optimum relative humidity of the air in the farm should be kept at 65-85% RH. Regarding the preferred conditions for mycelial growth, mycelium prefers dark condition to grow, but mushroom fruiting bodies prefer a bit of light. Mycelium consumes the nutrition in the substrate to grow. It expands to consume more nutrition in the neighboring areas of the substrate, and in this period, light is not necessary for the growth of mycelium. The higher density of mycelium combines with the sawdust particles, the lower the internal porosities in mycelium/sawdust composite are. Hence, greater strength can be anticipated.

Thus, to ensure homogeneous mycelium growth on sawdust and maximize the mycelium content, mushrooms with sawdust packages were stored in a laboratory container equipped with a smart farm system. The container was made from styrofoam, and holes were drilled into the lid to allow the air to circulate, and a black shading net covered the whole container to maintain dark condition for the incubating mycelium. The temperature in the laboratory was controlled at 26ºC using an air conditioner. A smart Arduino board was used to control the relative humidity of the air inside the container. A humidifier, temperature sensors, and relative humidity sensors were placed inside the container and connected to the smart board. The range of humidity was set up at 65-80% RH. If the relative air humidity became higher than 80%RH, the humidifier stopped spraying out water. This period usually took 7-10 days. Then, the sawdust package was broken on a surface cleaned with 95% alcohol inside the fume hood with its fan turned off. The sawdust containing the mycelium was molded into a rectangular block shape using polypropylene plastic molds of size 75 mm in width, 105 mm in length and 40 mm in height, for the uniaxial compression test, ASTM D3501-94. Cylindrical specimens were prepared using the same batch of mushroom spawn for the water absorption test, according to Appels et al. (2019). The specimens were shaped into cylindrical PLA plastic molds of 33.5 mm diameter and 25.50 mm height. The specimens were again incubated in the container where the dominant factors for the growth of oyster mushroom mycelium were controlled as closely as possible to the preferred condition using an Arduino smart board. The incubation period took 14-30 days. The specimen was demolded after the mycelium covered all the sawdust. According to Sharma et al. (2011), the thermal death point of *Pleurotus spp*. mushroom strain under dry conditions was 40-50ºC. Moreover, most fungi growing in competition with the mushroom mycelium, i.e., *Trichoderma spp*., cannot survive in temperatures higher than 50ºC (Sharma et al., 2007). There are several drying temperatures for MBC ranging from 60-130°C between a few hours to more than 24 h to remove the moisture in the MBC and inactivate the mycelium growth (Elsacker et al., 2020; Alemu et al., 2022; van den Brandhof & Wösten, 2022). The moisture in MBC should be kept as low as possible to prevent contamination. The mass of MBC should become stable during the drying process as it indicates that the maximum moisture content inside the specimens can be removed. Numerous works have reported that drying conditions of MBC range from 50-80°C within 24-46 h (Elsacker et al., 2020; Alemu et al., 2022). However, the dwell time per batch of the specimens is still too long. Ongpeng et al. (2020) used 110-115°C, not less than 24 h, to dry and prohibit mycelium growth of MBC, which has the mixing of sawdust, rice bran, and coconut husk as a substrate. Lelivelt (2015) reported that the mass of MBC, derived from wood chips and hemp, was constant when dried at 125°C for 2 h. Hence, the drying temperature for MBC in this study was set at 125°C to reduce the dwell time. Nevertheless, the contamination was found in MBC within 48 h at room temperature after MBC was dried at 125°C for 2 h. The mass of MBC in our cases was constant at 125°C after 4 h. Hence, the drying period was extended from 4 to 8 h to guarantee no contamination after the post-drying process of MBC. As a consequence, after the mycelium had covered the sawdust, the specimens were baked at 125ºC for 8 h using a convection oven (RedLINE, RF115, Germany). It was founded that the weight of all specimens after thermal deactivation at 125ºC for 8 h was constant, indicating that most of the moisture was removed. The weight of each sample was measured pre- and post-baking, and the percentage weight loss was recorded.

2.2 Physical properties measurement

2.2.1 Moisture content after deactivation of mycelium growth under heating

The percentage of weight reduction of MBC specimens after deactivation of the mycelium growth is an important factor affecting their properties because this indicates the remaining moisture content in the MBC specimens. The remaining moisture content in specimens directly enhances the creation of other fungi species grown on the specimens when stored at room temperature. According to ASTM D644 (ASTM International, 2002), the percentage of moisture content (W (%)) in the specimens can be calculated as follows:

$$W(\%) = \frac{W_1 - W_2}{W_1} \times 100\% \tag{1}$$

where W_1 and W_2 are weight of specimens before and after baking in grams, respectively.

2.2.2 Dry density

The mass of the MBC specimens was measured three times after baking (Kassa EK3840 digital scale, Thailand). The size of the specimens after baking was also measured three times to determine the volume. The density of PP/sawdust and PO/sawdust composites was determined as follows:

$$\rho = \frac{m}{V} \tag{2}$$

where ρ, m and V are the density [kg/m^3], mass of specimen after baking [kg] and volume of specimen after baking [m^3].

2.2.3 Water absorption

The cylindrical specimens obtained from the same batch of each as-received PO/sawdust and PP/sawdust composites with 33.5 mm diameter and 25.50 mm height were used for the water absorption test. Six specimens from each PO/sawdust and PP/sawdust composite were used in this experiment. It was found that the nominal weights of PO/sawdust and PP/sawdust composites before soaking in water were 4.86±0.15 g and 4.45±0.08 g, respectively. The test procedure for analyzing water absorptivity was modified from Appels et al. (2019). The specimens were immersed in distilled water in a beaker, taken out, and then the excess water was wiped out at the surface before their weights were measured (Metteler Toledo ML204 Analytical Balance, Switzerland) every 15 min for the first hour. Then, the weights of specimens were tracked after 4, 24, 48, 72 h. The final tracking was at 72 h. Therefore, there were nine times for tracking the weight of specimens during the test. The water absorption rate ($\dot{W}$) and the percentage of mass gain (W_{gain}) were calculated as follows:

$$\dot{W} = \frac{(W_n - W_0)}{t_n - t_{n-1}} \tag{3}$$

$$W_{gain}(\%) = \left(\frac{W_n - W_0}{W_0}\right) \times 100 \tag{4}$$

where W_n, W_0 and t_n represent the weight of specimen at the nth times [g], the weight of specimen before immersion in the distilled water [g], and the time of tracking the weight at nth times [s], respectively.

2.3 Compression test

Standard compression test methods for MBC were not available; however, as the MBC specimens in this study contained sawdust as the reinforcing phase with mushroom mycelium as the matrix phase. The standard compression test method for wood-based structural panels (ASTM D3501-94) was selected to test the PO/sawdust and PP/sawdust composites (ASTM International, 2000). After baking to deactivate the mycelial growth, it was found that the nominal width, length, and thickness of the PP/sawdust composite were 73.27±0.49 mm, 101.99±0.69 mm and 37.67±0.75 mm, respectively. The nominal width, length, and thickness of PO/sawdust composite were 73.36±0.96 mm, 101.73±0.87 mm and 36.77±0.46 mm, respectively. Six rectangular specimens of each PP/sawdust and PO/sawdust composite were compressed with a 2.5 mm/min strain rate at 23.7ºC, 53% RH, using a universal testing machine (Zwick/Roell, Z100SH, Germany). As the compressive load was applied, some sawdust particles came out from the side of specimens without fracture; the compression was stopped at 12 mm displacement, corresponding to 30% strain, and the maximum stress was recorded. The compressive modulus was determined from the slope of the initial stage of the linear stress-strain curve under compression. Interestingly, although crack initiation occurred, the specimens did not collapse, resulting in the changing in cross-sectional area of specimens under loading. The true stress (σ_{tr}) – true strain (ε_{tr}) was determined using equations (4) and (5), respectively.

$$\sigma_{tr} = \sigma_E \left(1 + \varepsilon_E\right) \tag{5}$$

$$\varepsilon_{tr} = \ln\left(1 + \varepsilon_E\right) \tag{6}$$

where σ_E and ε_E are engineering stress [kPa] and engineering strain [mm/mm].

2.4 Morphological observation

The morphology of mycelium inside the sawdust substrate and the outer skin was observed after compression test using a digital microscope with 2000X magnification (BM-DM61, Ningbo Barride Optics Co.,Ltd, China). The fracture area of specimens was observed and discussed.

2.5 Statistical analysis

Statistical analysis was carried out to analyze the potential significant differences between the mean values of the experimental data from the PO/sawdust and PP/sawdust composites. The experimental data from each of the two groups were analyzed using one-way analysis of variance (ANOVA) in Minitab (Minitab LLC., USA). The Tukey post-hoc test was performed to determine the significant level of mean values for each treatment. The experimental results are reported as the mean value along with the error bars calculated from the standard deviation, ±SD, of each group.

3. Results and Discussion

3.1 Physical properties

3.1.1 Moisture content after deactivation of mycelium growth and their dry density

After heating the specimens at 125ºC for 8 h, the growth of other fungi species, including mycelium, was deactivated. The moisture contained in the MBC specimens had evaporated during heating. The results confirmed that there was no remaining moisture in the specimens to create the growth of the other fungi species, leading to the contamination after the specimens were kept at room temperature. The weights of rectangular specimens in compression test and cylindrical specimens used for water absorption at pre- and post-deactivation was recorded. It should be noted that when similar type of materials is compared, larger specimens can store higher moisture content, consuming more time to remove all moisture rather than small specimens. Hence, the results of the moisture content after deactivation shown in Figure 2(a) are those obtained by heating the rectangular shape of MBC specimens used for compression test as their size and volume were larger than those of cylindrical-shaped specimens used for water absorption analysis. Figure 2(a) shows the moisture contents of PO/sawdust and PP/sawdust composites used for the compression test after deactivation of mycelium growth at 125ºC for 8 h. Notably, the weights of both PO/sawdust and PP/sawdust composites for compression test after deactivation mycelium growth were reduced by 58-68%. Moreover, the percentages of weight reduction of the specimens used for water absorption test were similar, in the range of 55-65%, which was in line with the range for rectangular MBC specimens. Consequently, it can be implied that the weight reduction of MBC specimens after deactivation was independent of their size and volume. The substrate type is considered as the main factor influencing the removal of moisture content in MBC when similar conditions are used to deactivate mycelium growth. Figure 2(b) shows the dry densities of PP/sawdust and PO/sawdust composites. The average densities of the PP/sawdust and PO/sawdust composites were 292.14 and 272.17 kg/m^3, respectively. The average density of the PP/sawdust was slightly higher than PO/sawdust composite. It was found that the density of mycelium composites in this study was within the density range of oyster mushroom mycelium/sawdust composite (100-270 kg/m^3) mentioned in several literatures (Islam et al., 2017, 2018; Elsacker et al., 2018; Appels et al., 2019; Elsacker et al., 2020). Generally, the density of a mycelium-based composite comes from the density of the mycelium that binds the particulate substrate together. Hence, the digital microscope is necessary to observe the outside appearance and inside morphology of specimens after compression.

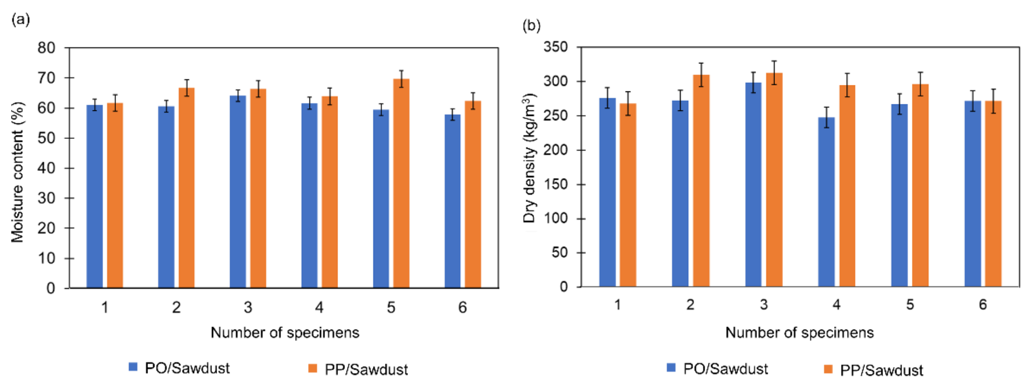

Figure 2. (a) Moisture content of PO/sawdust and PP/sawdust composites used for compression test after deactivation the growth of mycelium at 125ºC, 8 h and (b) The dry density of PO/sawdust and PP/sawdust composites. The standard deviation, ±SD, is plotted as error bars.

3.1.2 Water absorption characteristic

The weight of the cylindrical MBC specimens used for water absorption in pre- and post-baking along with the percentage of mass reduction are shown in Table 1. According to Table 1, the nominal weights of PO/sawdust and PP/sawdust composites after deactivation by heating were 4.97±0.48 g and 7.47±0.47 g, respectively. The average percentage of

Table 1. The weight of mycelium growth of MBC specimens used for water absorption test in pre-, post-baking and the percentage of weight reduction

Sample Number	Pre-baking Weight (g)	Post-baking Weight (g)	Weight Reduction (%)
PO/sawdust #1	18.57	4.64	75.01
PO/sawdust #2	21.12	5.52	73.86
PO/sawdust #3	20.81	5.64	72.90
PO/sawdust #4	18.46	4.53	75.45
PO/sawdust #5	19.24	5.10	73.49
PO/sawdust #6	16.84	4.40	73.89
PP/sawdust #1	17.08	6.66	61.03
PP/sawdust #2	19.07	7.79	59.15
PP/sawdust #3	19.58	8.01	59.09
PP/sawdust #4	19.13	7.32	61.74
PP/sawdust #5	18.33	7.87	57.06
PP/sawdust #6	19.16	7.15	62.66

weight reduction of PO/sawdust composites, 74.10%, was higher than PP/sawdust composites, 60.12%. It should be noted that there was no contamination observed in the specimens when they were stored at room temperature.

Figure 3(a) shows the specimen mass gain as a function of time in hours. The slope of PP/sawdust composites gradually increased in the first 30 min, followed by a moderate decrease from 30 min to 4 h. Then, the slope was flat and close to zero from 24 h to 72 h, indicating the limitation of water absorption capacity of PP/sawdust composites. At 72 h, it was observed that the average percentage of mass gain due to water absorption into the PP/sawdust composite was 203.44±11.49%. Additionally, six specimens of PP/sawdust and PO/sawdust composites were still floating in the water. On the other hand, the characteristic slope of PO/sawdust composite was completely different under water absorption test. The slope of PO/sawdust composite was considerably lower within the first four hours compared to PP/sawdust composite. Notably, the slope of PO/sawdust composite rapidly increased from 1-24 h and then became small and constant after 24 h. The specimen mass gain at 72 h of the PO/sawdust composite was 144.04±13.89%, 1.41 times lower than the PP/sawdust composite. The smaller slope of specimen mass gain suggested less water absorption into the specimen. As a result, it can be implied that PO/sawdust composite absorbed water at a slower rate than the PP/sawdust composite, which was supported by the findings of Attias et al. (2017). They reported that the *P. pulmonarius* mycelium-based lignocellulosic substrate retained a higher amount of water during the experiment compared to other mycelium species. Figure 3(b) shows the water absorption rate as a function of time for the PO/sawdust composite and PP/sawdust composite. According to Figure 3(b), the fastest water absorption rate was found in the first 15 min, and the rate of water absorption became zero after 24 h for both types of specimens, indicating that there was no water absorption into PO/sawdust and PP/sawdust composites after 24 h. The highest water absorption rate of PP/sawdust composite was 5.12±2.01 mg/s in the first 15 min, while the water absorption rate of PO/sawdust composite was 0.67±0.32 mg/s in the same period. The water absorption performance of MBC is attributed to the hydrophobic properties of mycelium as it contains protein hydrophobins at its surface, resulting in a high level of water-repellence (Kohphaisansombat et al., 2023). Based on our findings, the water absorption performance of MBC was influenced not only

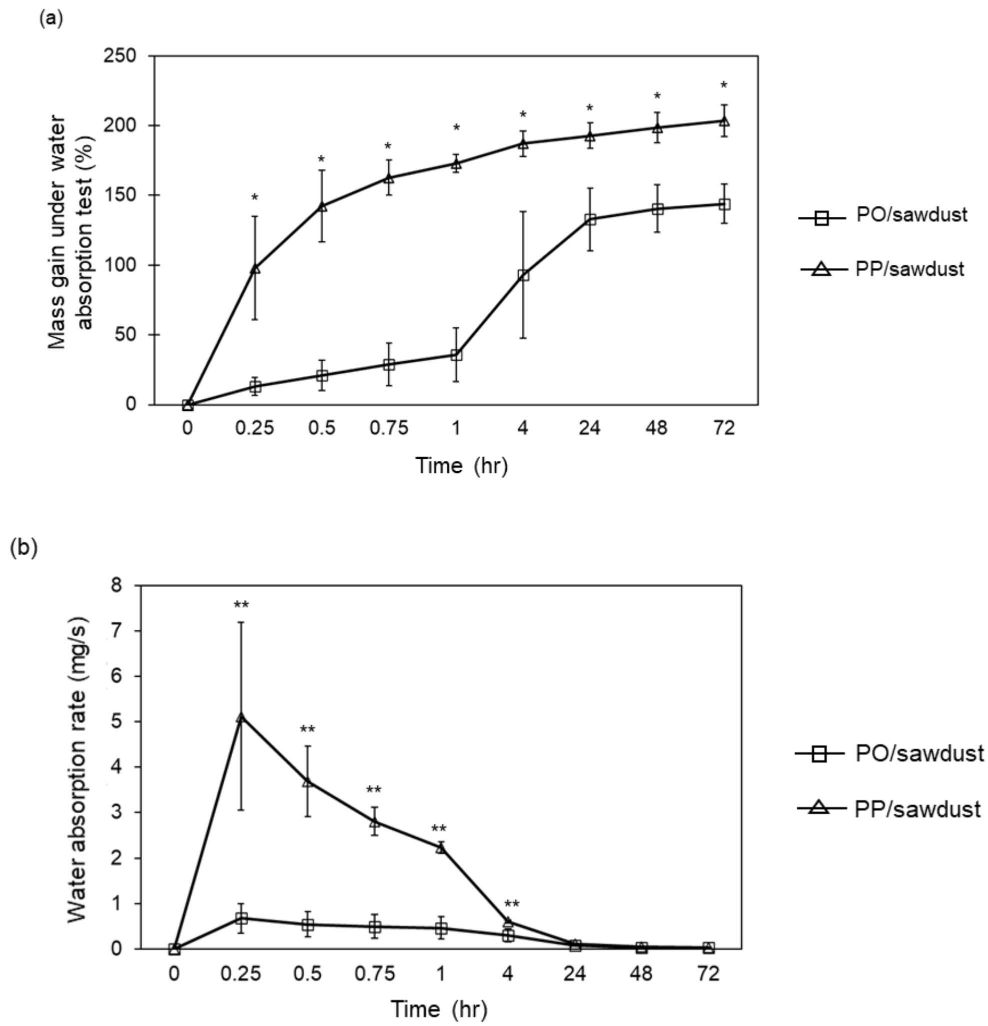

Figure 3. Water absorption characteristic of PO/sawdust and PP/sawdust composites: (a) percentage of mass gain and (b) water absorption rate as a function of time. Values presented are the mean±SD of six replicates. "*, **" presents a significant difference between PO/sawdust and PP/sawdust composites based on Tukey post-hoc test at the significance level P < 0.05 and 0.1, respectively.

by its hydrophobic characteristics but also by the morphology of mycelium. Although the mycelium belongs to the same species, *Pleurotus* spp., the morphology of each fungal species is different, leading to different water absorption properties. The mycelial morphology of *P. ostreatus* and *P. pulmonarius* was observed and the results regarding the water absorption performance and mechanical properties are elaborated in the next section. Besides, it should be noted that no contamination was observed at the surface of MBC specimens when they were immersed in water during and after finishing the test.

3.2 Mechanical properties

Six specimens in each PO/sawdust and PP/sawdust composite were tested under compression according to ASTM D3501-94. The weights of the rectangular MBC specimens used for compression test in pre- and post-baking to deactivate the mycelium growth are provided in Table 2.

Table 2. The weight of mycelial growth of MBC specimens used for compression test in pre-, post-baking and the percentage of weight reduction

Sample Number	Pre-baking Weight (g)	Post-baking Weight (g)	Weight Reduction (%)
PO/sawdust #1	193.20	77.10	60.09
PO/sawdust #2	188.20	74.20	60.57
PO/sawdust #3	218.20	78.20	64.16
PO/sawdust #4	174.60	68.97	60.49
PO/sawdust #5	171.40	72.10	57.94
PO/sawdust #6	184.00	77.29	57.99
PP/sawdust #1	201.00	77.00	61.69
PP/sawdust #2	250.00	83.30	66.68
PP/sawdust #3	257.50	86.50	66.41
PP/sawdust #4	225.50	81.40	63.90
PP/sawdust #5	257.00	77.90	69.68
PP/sawdust #6	193.80	73.00	62.33

Table 2 shows that the nominal weights of PO/sawdust and PP/sawdust composites samples for compression test were 74.64±3.28 and 79.85±4.42 g, respectively. The average percentages of reduction of PO/sawdust and PP/sawdust composites were 60.21% and 65.12%, respectively, which were within the range of weight reduction of MBC specimens used for the water absorption test, 60.12-74.10%. Hence, it can be implied that the contamination could not be found in the specimens with weight reduction in 60-75% probably due to low moisture content in specimens. Moreover, the weight reduction after baking at 125ºC, 8 h was independent of the sample size.

The average of true stress-true strain curves of PO/sawdust and PP/sawdust composites was determined and plotted along with error bars as shown in Figure 4. An inset in Figure 4 shows the average compressive modulus of PO/sawdust composites under compression of 0.81 MPa, and the PP/sawdust composites, 0.86 MPa, determined from the slopes of the average true stress-true strain curves in the elastic region. Although the mean value of the stress of PP/sawdust composites was slightly higher than the PO/sawdust composite, the mean values of maximum compressive stress at 30% strain of both groups were similar at 1.13 MPa. Furthermore, the statistical analysis disclosed that there were no statistically significant differences at the 95% confidence level between the compressive moduli of the PO/sawdust and PP/sawdust composites under compression test. Consequently, it can be deduced that the compressive moduli of PO/sawdust and PP/sawdust composites were equivalent.

The PO/sawdust composite showed a wider error bar than the PP/sawdust since there was a larger variance of experimental data when compared to PP/sawdust composite. Specimen no.2 (PO/sawdust#2) from six specimens exhibited a remarkably low compressive modulus, 0.5 MPa, compared to the other MBCs in the test. However, it had

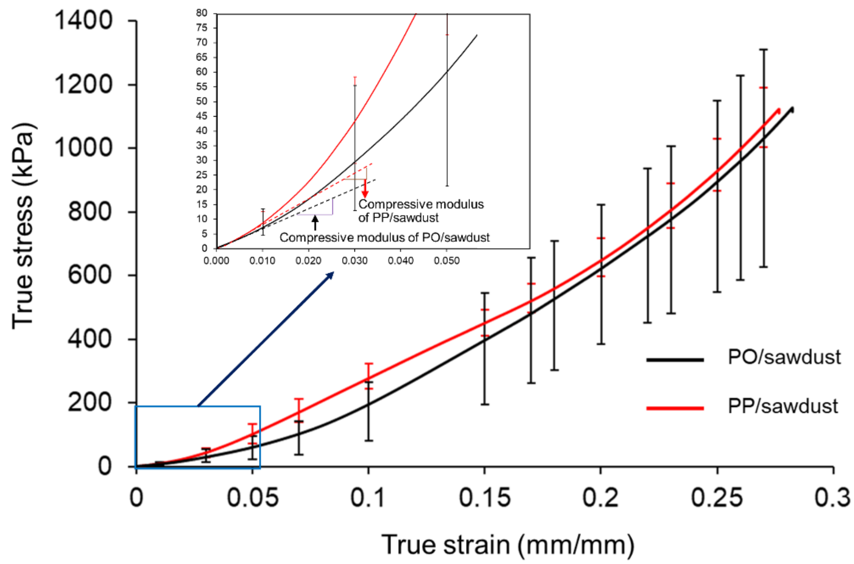

Figure 4. True stress – true strain curves of PO/sawdust and PP/sawdust composites. Data are plotted in average value along with error bars determined from standard deviation.

the highest density, 298.61 kg/m^3, of the PO/sawdust composite group. Meanwhile, among the group of PP/sawdust composite, specimen no.2 (PP/sawdust#2) and specimen no.3 (PP/sawdust#3) contained higher than average density values, 309.59 kg/m^3 and 313.03 kg/m^3, respectively, and their compressive moduli were 1.34 MPa and 0.83 MPa, respectively. Therefore, the greater density of oyster mushroom mycelium/sawdust composite did not contribute to the higher compressive modulus of MBC. The density of mycelium-based composite specimens could come from the dense area of the mycelium, forming a three-dimensional network with the sawdust inside and outside the specimens. Morphological observation after the compression test was necessary to better explain the mechanical behavior of PO/sawdust and PP/sawdust composites under compression. The highest and lowest compressive moduli in each group of PO/sawdust and PP/sawdust composites were selected for morphological observation to investigate the morphology of both composites and their relationship with mechanical properties. The inner and outer skins of specimens after the compression test were observed using a digital microscope. Note that the specimens with the highest and lowest compressive moduli in individual groups were labelled #1 and #2, respectively.

Figure 5(a-h) shows the digital microscopic images of the outer and inner skins of the PO/sawdust#1, #2 and PP/sawdust#1, #2. These images reveal significant differences in the mycelium morphology of *P. ostreatus* and *P. pulmonarius*. The mycelium of *P. ostreatus* was denser and fluffier with a cotton-like appearance, while the mycelium of *P. pulmonarius* was less dense with root-like and dendritic growth. Figure 5(a-b) indicates that the mushroom mycelium (white skin) mostly covered the sawdust particles (brown) on the outer skins of PO/sawdust#1 and PP/sawdust#1, respectively. In contrast, more sawdust particles (brown) appeared on the outer skin of PO/sawdust#2 (Figure 5c) and PP/sawdust#2 (Figure 5d). Moreover, Figure 5(g, h) suggest that the mycelium grew on

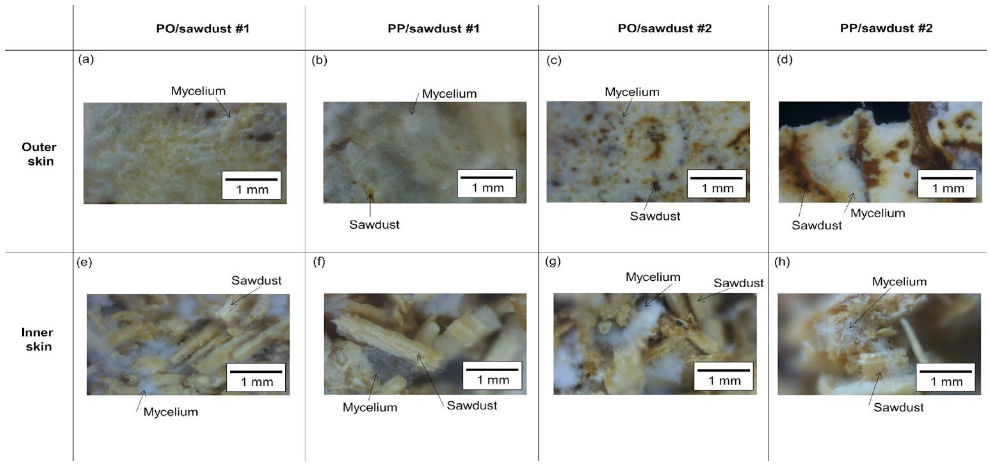

Figure 5. Digital microscopic images of outer and inner skin after compression test of (a, e) PO/sawdust #1, (b, f) PP/sawdust #1, (c, g) PO/sawdust #2 and (d, h) PP/sawdust #2

sawdust with less homogeneity at the surface of PO/sawdust#2 and PP/sawdust#2 compared to the inner skin of PO/sawdust#1 (Figure 5e)) and PP/sawdust#2 (Figure 5f). Figure 5(e) shows the densest mycelium combining the sawdust particles inside PO/sawdust#1 compared to the other specimens (Figure 5(f-h)). Higher air porosity with thinner and root-like mycelia was observed in PP/sawdust#2 (Figure 5(h)) compared to PO/sawdust#1 (Figure 5(e)).

Appels et al. (2019) reported that mycelium skin fosters the waterproof properties of MBC. Based on the morphology of mycelium of *P. ostreatus*, which was dense and cotton-like, it can be deduced that the dense cotton-like mycelium promoted the ability of waterproof more than the less dense root-like morphology of *P. pulmonarius*. PO/sawdust#1 and PP/sawdust#1 exhibited the highest compressive modulus of 1.34 MPa in each group of PO/sawdust and PP/sawdust composites. However, their fracture behaviors under compression were different and this could be explained by the different mycelium morphology of *P. ostreatus* and *P. pulmonarius*. The dense, fluffy and cotton-like mycelium in *P. ostreatus* led to the formation of strong shell mycelium, while the root-like and more homogeneous thickness formed robust core mycelium of *P. pulmonarius* (Kuribayashi et al., 2022). The shell mycelium consisted of a thin layer of spongy mycelium that formed at the outer surface of the specimen by binding with the in-plane hyphae, whereas the core mycelium penetrated inside and constructed a three-dimensional network with the sawdust. Figure 6(a, c) shows that the fracture occurred at the side surfaces of PO/sawdust#1 and some had not collapsed. Under the compression test, a specimen generally expands in the direction perpendicular to compressive load and contracts along the direction uniaxial with the compressive load. It is noteworthy that the surface of PO/sawdust#1 still remained in a shell shape at the outermost surface, indicating that the shell mycelium played an important role in preventing the fracture of PO/sawdust#1. Figure 6(b,d) shows the top and front views of PP/sawdust#1 after the compression test, indicating that the side surfaces of PP/sawdust#1 broke continuously but

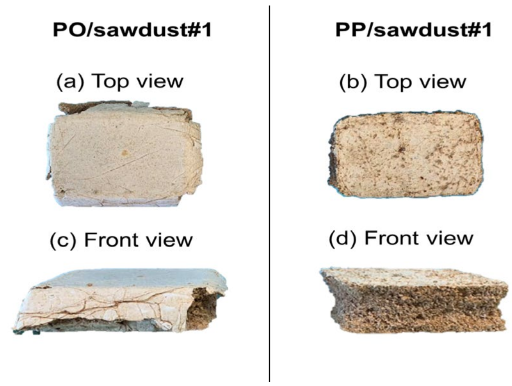

Figure 6. Fracture characteristics of MBC specimens after the compression test: (a, c) Top and front views of PO/sawdust#1 and (b, d) top and front views of PP/sawdust#1

there was no fracture at the core of specimen. Thus, unlike PO/sawdust#1, the shell mycelium was unable to resist the compressive load in the PP/sawdust#1, but the core mycelium did. This suggested that the root-like morphology of mycelium found in *P. pulmonarius* formed a robust core mycelium inside the specimen, while the cotton-like mycelium of *P. ostreatus* formed shell mycelium by binding the sawdust particles at the planar surface. The robust core mycelium resulted from the homogeneous and root-like mycelium was able to slightly enhance the higher mean of compressive stress observed in PP/sawdust composite (Figure 4). Thus, it can be deduced that the distinction in mycelium morphology leads to different failure modes under compression. These findings underscore the profound implications of mycelium morphology for mechanical properties.

Moreover, several works reported that different fungal species and mycelium morphology grown on the substrates greatly influenced MBC properties (Aiduang et al. 2022a; 2022b). According to Kuribayashi et al. (2022), *P. ostreatus* formed a coarse cotton-like mycelium of heterogeneous thickness on agar plates, while *T. hirsute* formed dense and thick mycelium. Pham et al. (2023) found that *P. pulmonarius* formed root-like mycelium on agar plates. These works supported our morphological observation of mycelia grown on sawdust, as shown in Figure 5. In addition, to observe the mycelium growing characteristics, the growth of *P pulmonarius* and *P. ostreatus* mycelia on millet, which was sold as the master spawn of mushrooms, was observed as shown in Figure 7. The fluffy and cottony mycelia of *P. ostreatus* was clearly observed in the master spawn (Figure 7(a)), while the rooting with uniform expansion was seen in *P. pulmonarius* (Figure 7(b)). Furthermore, a lower degree of non-homogeneous growth and thicker mycelium was obviously seen in *P. ostreatus* compared to *P. pulmonarius*.

Figure 8(a) shows the compressive moduli of PO/sawdust and PP/sawdust composites as a function of density compared to the other mycelium spawn types grown on different lignocellulosic substrates: TV/Flax treated tow, TV/Chopped hemp, TV/Wood loose from Elsacker et al. (2018). This indicates that the PO/sawdust and PP/sawdust composites in this study exhibited compressive moduli and densities within the range of 250-320 kg/m^3. The densities of the PO/sawdust and PP/sawdust composites were relatively higher than those of the three samples: TV/Chopped hemp, TV/Wood loose, TV/Flax treated tow, from the reference (Elsacker et al., 2018). The TV/Chopped hemp, TV/Wood loose, and TV/Flax treated tow composites exhibited 0.77, 0.14, 0.41 MPa compressive moduli, respectively, while their dry densities were 87.4, 97.4 and 187 kg/m^3,

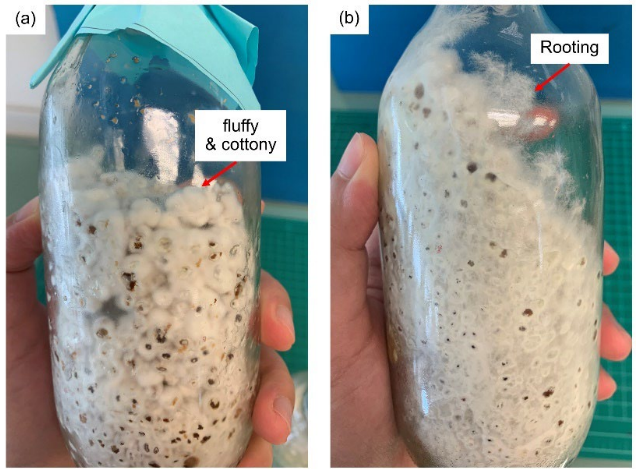

Figure 7. Master spawn purchased from the same mushroom farm: (a) Fluffy and cottony *P. ostreatus* mycelium and (b) Rooting *P. pulmonarius* mycelium.

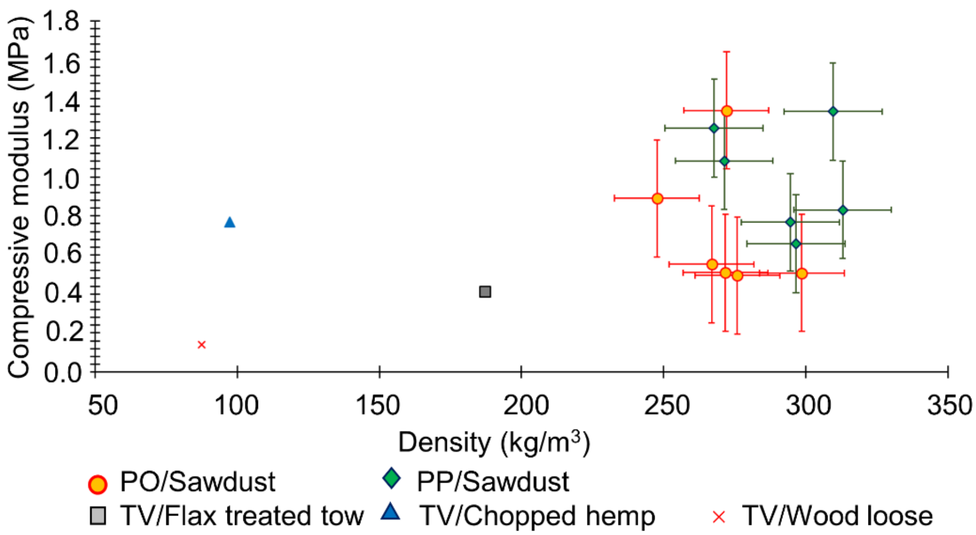

Figure 8. Compressive modulus under compression test of PO/sawdust and PP/sawdust composites as a function of density compared to the other mycelium spawn types in different lignocellulose substrates: TV/Flax treated tow, TV/Chopped hemp, TV/Wood loose from Elsacker et al. (2018). Mycelium spawn: TV: *Trametes versicolor*.

respectively. The wood loose had not been chopped and was used to fabricate MBC without any processing, but the chopped fibers were sieved with a 5 mm strainer. Therefore, when similar families of mycelium are compared, a smaller substrate size enhances the compressive modulus. The size of sawdust particles in this study was not sieved; however, their size was larger than 3 mm, as shown in Figure 5. Hence, the size of sawdust particles was comparable to chopped hemp fiber used in Elsacker et al. (2018), which had a compressive modulus that was within the average values of the PO/sawdust and PP/sawdust composites in this study.

There are several properties that should be considered for selecting MBC as packaging materials: the remaining moisture content, density, compressive modulus, compressive strength and water absorption performance. In this study, the average moisture contents after deactivation of PO/sawdust and PP/sawdust composites were 60.78±1.95% and 65.12±2.76%, respectively, which corresponded with those of the mushroom mycelium-based agricultural wastes (Aiduang et al., 2022b, 2024). It is noteworthy that these amount of moisture contents were insufficient to generate contamination on the PO/sawdust and PP/sawdust composites when the specimens were stored at room temperature. The average weight of PP/sawdust composite before deactivation by heating was 230.8 g, which was greater than PO/sawdust composite, 190.86 g. This indicates that *P. pulmonarius* stored higher moisture and required higher humidity in the substrate for mycelial growth than *P. ostreatus*. Hence, a greater moisture content remained in PP/sawdust composites after heating in a convection oven. The densities of PO/sawdust and PP/sawdust composites were in the range of 250-310 kg/m^3, which was close to the density of *Pleurotus ostreatus*/sawdust composite, 178.50-552.0 kg/m^3, seen in a previous study (Aiduang et al., 2022a). Moreover, the densities of both PO/sawdust and PP/sawdust composites in our study matched with the lower bound of the density of rigid polyurethane foam (283-300 kg/m^3) (Lifshitz, 1983; Mane et al., 2017). This suggested that *P. pulmonarius* can be an alternative choice to *P. ostreatus* mycelium grown on sawdust as a substitute for packaging made from low-density polyurethane foam.

Nevertheless, the compressive strength of low-density polyurethane foam, 7.5 MPa (Lifshitz, 1983), is relatively higher than PO/sawdust and PP/sawdust composites, which have maximum strength of 1.34 MPa at 30% compression. Therefore, if a similar level of compressive strength is preferred, the strengths of the PO/sawdust and PP/sawdust composites should be improved. The compressive moduli of the PO/sawdust and PP/sawdust composites under compression were equivalent and in the range of 0.5-1.40 MPa (Figure 8), which was in good agreement with the compressive moduli of mushroom mycelium/red oak sawdust particles, 1 MPa, reported in previous literature (Jones et al., 2020). Based on the compressive modulus and strength of our MBC, the results indicated that their stiffness and strength were at a similar level to the non-recyclable plastics: phenolic formaldehyde resin foam (0.2-0.5 MPa) and extruded polystyrene foam (0.2-0.7 MPa) (Aiduang et al., 2024). Notably, the compressive strain of our PO/sawdust and PP/sawdust composites was higher than the compressive strain of low-density polyurethane (15-26%) (Lifshitz, 1983; Mane et al., 2017) and phenolic formaldehyde resin foam (0.2-15.7%) (Aiduang et al., 2024) probably because of the mycelium-sawdust network strengthening the MBCs from different mycelium morphology. Based on the experimental results, the sawdust particles emerged slightly from the MBC specimens without breakage under compression. Therefore, compressive strain was continuously applied to the PO/sawdust and PP/sawdust composites up to 30% strain. Consequently, this distinct fracture characteristics of MBC promoted its higher ductility compared to non-recyclable foam packaging.

Typically, the water absorption of mushroom mycelium based-agricultural-composites was reported at 200-278.9% (Aiduang et al., 2022a). According to our water absorption results, it is noteworthy that the PO/sawdust and PP/sawdust composites contained a high degree of porosity, resulting in weight gain under water immersion up to the maximum of 203% after 72 h in the case of the PP/sawdust composite. During water absorption, a higher number of open pores allows more water to flow inside the specimen, while closed pores can resist water flowing into the specimen. In addition, non-homogeneous mycelial growth could lead to the nonuniform density at the local region of the specimen, resulting in the formation of closed and open pores in the MBC specimens. It was reported that the mycelium delayed the penetration of water into MBC; however, the water absorption capability was shown to have come from the substrate (Kuribayashi et al., 2022). The slow water absorption rate found in the PO/sawdust composite was attributed to the coarse, fluffy, and cottony shell mycelium which constructed a strong network between *P. ostreatus* mycelium and sawdust particles at the outermost surface of specimens. On the other hand, the root-like mycelium found in *P. pulmonarius* allowed water to penetrate into specimens 7.5 times faster than *P. ostreatus* mycelium. The root-like mycelium did not form a strong network with sawdust particles at the specimen surface; therefore, the shell mycelium of *P. pulmonarius* allowed more water to penetrate through the gap of the shell mycelium. Nevertheless, the root-like mycelium was able to penetrate and grow uniformly to form strong core mycelium, contributing to a slightly higher compressive modulus under compression test in the case of the PP/sawdust composite. As a consequence, when the water absorption capability is considered, the mycelium of *P. ostreatus* would be the better option.

4. Conclusions

The compressive modulus, density, and water absorption of oyster mushroom mycelium (*Pleurotus ostreatus*)/sawdust composite and Indian oyster mushroom mycelium (*Pleurotus pulmonarius*)/sawdust composites, were investigated. Indian oyster mushrooms are likely found in Thailand, China, and New Zealand and prefer warmer weather than other oyster mushrooms. Therefore, Indian oyster mushrooms are commonly found, are highly abundant and are of lower price in tropical countries. Based on our experimental results, it can be confirmed that the mushroom mycelia of *Pleurotus* spp., exhibited equivalent compressive moduli in the range of 0.8-1 MPa. Moreover, the densities of the PO/sawdust and PP/sawdust composites were within the range of 250-310 kg/m^3, a similar range to low-density polyurethane foam used for packaging materials.

Nevertheless, the compressive strengths of the PO/sawdust and PP/sawdust composites need to be improved if they are to substitute for polyurethane foam. The morphology of mycelium network binding with sawdust particles mainly contributed to the strength and ductility of both MBCs in this study since they could sustain up to 30% compressive strain, which is higher than low-density polyurethane foam and phenolic formaldehyde resin foam. Moreover, the digital microscopic images revealed that the morphology of the mycelia of each fungal species resulted in different failure modes under compression as well as different retardation of water absorption. The fluffy and cottony shell mycelium mostly occupied the outermost surface of *P. ostreatus* mycelium/sawdust composite prevented the surface of PO/sawdust composite from failure and delayed water penetration into specimens compared to PP/sawdust composite. The fine and root-like *P. pulmonarius* mycelium expanded throughout and grew uniformly on sawdust to form strong core mycelium inside the specimen. Hence, a slightly higher compressive modulus was found for the PP/sawdust composite, and the surface of the specimens tended to fracture but the core of the specimens did not. Furthermore, the root-like mycelium allowed more water to penetrate through the gaps of the shell mycelium into the core mycelium. Therefore, if high water resistance was preferred for packaging, the PO/sawdust composite would be a better choice than PP/sawdust composite.

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6. Conflicts of Interest

The authors declare no conflict of interest with respect to the research, authorships, and/or publication of this article.

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