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The Potential of Mycofoam as a Biocomposite Material with Various Substrate and Mushroom Compositions

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Mycofoam is a biocomposite composed of mycelial filaments that bind to the host substrate, which can replace polystyrene. This research aims to determine the most appropriate formulation of the growing medium and mushroom to be used in mycofoam products based on some parameters. Composition is one of the main factors for selecting the most appropriate mycofoam to replace polystyrene. On the other hand, the quality of mycofoam can be assessed through its strength and water resistance. The mushroom mycelium used in this study came from *Pleurotus ostreatus* and *Lentinula edodes*. Besides, the growing medium formulation comprises five different compositions, each containing different proportions of sawdust and bagasse. This research was composed of preparation of growing medium, spawn inoculation, molding, heating, testing, and data analysis. Based on the results, the combination of 100 percent sawdust and *L. edodes* was the most appropriate choice compared to other formulations based on strength and appearance. The results of the water absorption test showed that all mycofoam formulations were not resistant to water, bio-based coating can be used to overcome this problem.

Key words: Mycofoam, formulations, mushroom mycelium, substrate

INTRODUCTION

Polystyrene, commonly known as styrofoam, is a synthetic aromatic polymer made from styrene monomers with high molecular weight (Ho *et al.* 2017). Polystyrene has many properties, such as pressure resistance, lightweight, and thermal insulation (Liang *et al.* 2021). Moreover, polystyrene covers many types such as Oriented Polystyrene (OPS), High Impact Polystyrene (HIPS), and Expanded Polystyrene (EPS) foam. EPS has become the most widely used (Ho *et al.* 2017). However, EPS and other polystyrene waste will be difficult to decompose and handle (Palmer *et al.* 2022). In Indonesia, polystyrene has become an important issue that should be seriously resolved. Lembaga Ilmu Pengetahuan Indonesia (2018) found that a total of 0.27 to 0.59 million tons of garbage entered the Indonesian Sea and was dominated by polystyrene (Fajar 2019). According to the US Environmental Protection Agency (EPA), the manufacturing process for polystyrene produces the 5th largest hazardous waste in the world (Farrelly and Shaw 2017). Therefore, it is necessary to have a product innovation that can substitute polystyrene.

Mushroom mycelium has the ability as a natural glue that sticks to organic substrates, which will form a solid and hard structure to resemble polystyrene (Abhijith *et al.* 2018). The substitution product of polystyrene in the form of a biocomposite from mushroom and organic substrates is called mycofoam. Mycelium will develop and produce many self-assembling bonds in the form of fibers that cover the surface of the substrate, digest it, and bind it to become stronger (Sivaprasad *et al.* 2021). Mycelium that grows on this organic substrate will stop growing with the heating treatment and then create mycofoam. Hopefully, this natural substitute for polystyrene will be easier to decompose in a few weeks (Abhijith *et al.* 2018). The use of this biocomposite has also been commercialized by the Ecovative Design Company in recent years, but this product still needs to be reviewed to get the right composition to be marketed globally (Kim & Ruedy 2019). In research conducted by Aquino *et al.* 2022, differences in mushrooms can affect the final function of mycofoam products. The composition of the substrate also plays a significant role in influencing mycelium growth in mycofoam (Peng *et al.* 2023).

As the main reference to improve the quality and quantity of mycofoam, the selection of the main components such as mycelium can be developed

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based on strength and abundance in nature. Some mushrooms that are likely to have strong mycelium based on their appearance and easily grow in Indonesia such as *Pleurotus ostreatus* (oyster mushroom) and *Lentinula edodes* (shiitake mushroom) (Saskiawan and Elfarisna 2019; Putra *et al.* 2023). The mycelium in these mushrooms contains chitin which can provide tensile strength to fibrous tissues such as organic substrates (Ongpeng *et al.* 2020). *P. ostreatus* and *L. edodes* can colonize well and grow rapidly if they get the right substrate (Tacer-Caba *et al.* 2020). These two species of mushrooms can degrade lignin, cellulose, and hemicellulose in wood because they are classified as white-rot fungi that have lignocellulolytic enzyme activities (Kuijk *et al.* 2016). Besides, some of the most commonly used substrates as mushroom growing medium in Indonesia are sawdust and bagasse (Siregar *et al.* 2020). In addition to reducing polystyrene waste, this product can also reduce organic substrate waste which is usually produced by the wood industry. This research aims to determine the most appropriate formulation of the growing medium and mushroom to be used in mycofoam based on some parameters, such as strength, water resistance, and appearance.

MATERIALS AND METHODS

Preparing Growing Medium with Various Substrate Formulations. The preparation of the growing medium was divided according to the formulations in Table 1. The growing medium consisted of 89 percent of the main substrate, 9 percent of rice bran, 1.5 percent of $CaSO_4$, and 0.5 percent of $CaCO_3$ (Khusnul *et al.* 2021). All the ingredients were mixed until homogenous and then added to water gradually. The growing medium was coagulated in the hand and measured qualitatively until the substrate did not dissolve to determine whether the water content was sufficient. Then, the mixture was put into a polypropylene heat-resistant plastic number 08 with a size of 30 × 40 cm. The growing medium was sterilized in an autoclave for 45 minutes at a temperature of 121°C and a pressure of 1 atm. The autoclave used is Hirayama HICLAVETM HVE-50.

Inoculation and Observation of Mycelium in Growing Medium. After being left to warm conditions, the growing medium was inoculated with the commercial mushroom spawn of *P. ostreatus* and *L. edodes* which had been obtained from "Bibit Jamur Sumedang, Indonesia" cultivators. Inoculation was done by using a spatula to take the mushroom spawn in the bottle, then the spawn was put into a growing medium plastic aseptically. The mixture of growing medium and mushroom spawn was stored in a dark place at room temperature of 25-27 °C until the mycelium filled the growing medium (Kishan *et al.* 2018; Santhosh *et al.* 2018). Inoculation to the growing medium has been done seven times. To accurately determine the growth rate, the incubation of the mushroom mycelium in each formulation of the growing medium was also carried out on a small scale using a 140 ml bottle and 5 g of mushroom spawn. Repetitions were carried out three times and observations to collect the data were carried out every day until the mycelium filled the growing medium in the bottle (Figure 1).

Mycofoam Molding. After the mycelium filled the growing medium, the entire contents of the growing medium were transferred to a sterile tub. Then, the growing medium was kneaded to avoid clump. A total of 30 g/kg corn starch was added into the growing medium to help the mycelium grow back after squeezing (Syawal *et al.* 2019). Afterward, the growing medium that had not clotted was spread without pressure into a mold with a size of 20 × 10 × 2.5 cm. The mold was covered with plastic wrap and small holes were made using sterile skewers. Molding has been done seven times. Then, it was re-incubated in the dark at room temperature until the mycelium filled the growing medium again (Santhosh *et al.* 2018).

Mycofoam Heating. When the mycelium covered the entire surface of the growing medium in the mold, the mycofoam was ready to be heated. Before putting it into the oven, the mycofoam was removed from the box and weighed before heating (M1) and put in the oven at 100°C (Santhosh *et al.* 2018). The oven used is Memmert UNB 500. Then, the weight of mycofoam was re-checked after the heating (M2). Mycofoam was declared dry if the water content was reduced by at least 65 percent.

Table 1. The code of the various growing medium formulations

	P1R (%)	P2R (%)	P3R (%)	P4R (%)	P5R (%)
Sawdust	100	70	50	30	-
Bagasse	-	30	50	70	100

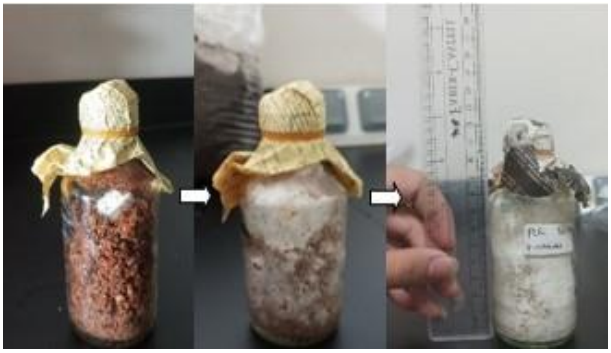

Figure 1. Mycelium observation on a small scale

Mycofoam Strength Analysis. The compression test was done to determine the compression strength of the heated mycofoam. This test was carried out using the RME 100 compression machine at the Laboratorium Teknologi Kekuatan Struktur (LTKS), Puspitek. One sample of mycofoam was placed on a flat surface provided horizontally, then given an axial load until the final height of the mycofoam was reduced by half. The compression test used the following formula:

$$CS = \frac{F}{A}$$

Where:
CS = compressive strength (Kgf/cm^2)
F = compressive load (Kgf)
A = mycofoam Dimension (cm^2)

Mycofoam Water Resistance Analysis. This analysis was carried out to check the amount of water absorbed by mycofoam. Mycofoam which has been heated at the oven stage was cooled at room temperature and the 2nd mass (M2) was obtained. Mycofoam must be completely dry before being soaked in clean water at room temperature of 25-27°C for 24 hours. Mycofoam was removed and the remaining water was cleaned with a dry tissue. Then, it was weighed and the 3rd mass (M3) was obtained. This test was carried out three times. Water absorption was calculated using the following formula:

$$W = \frac{(M3 - M2)}{M2} \times 100$$

Where:
W = water absorption (%)
M2 = mycofoam's mass after heating (g)
M3 = mycofoam's mass after soaking (g)
(Kishan *et al.* 2018)

Statistical Analysis. Statistical analysis was performed using IBM SPSS Statistics version 23. All data with repetitions were tested for normality using Kolmogrov-Smirnov and homegeneity test using Levene's test. After passing the normality and homogeneity tests, one-way ANOVA test was carried out to see the difference between the formulations and two-way ANOVA to see the interaction differences between the formulation and mycelium, which was then followed by a Tukey post-hoc test. If the data is not normally distributed and not homogeneous, then the Kruskal-Wallis test was on the formulation data or the Friedman test on the formulation and mycelium interaction data was performed. Statistical tests were considered if $p<0.05$.

RESULTS

Mycelium Growth on Growing Medium. Growing medium were added with mushroom inoculums of *P. ostreatus* and *L. edodes*. Observations were made once a week for 3 to 5 weeks and the results were shown in Figure 2. Based on the mycelium growth observation result, *P. ostreatus* and *L. edodes* can grow well in the five formulations of growing medium. However, when the growth time was observed, the mycelium of *L. edodes* tended to take longer to fill the growing medium than *P. ostreatus.* To accurately determine the growth time of the mycelium, the test was repeated on a small scale with three repetitions. The results are listed in Figure 3. Based on the result, the fastest growth of mycelium is in the P3R medium formulation in both types of mushrooms. In comparison with mycelium growth rate, *P. ostreatus* is faster than *L. edodes*. Based on Friedman's non-parametric test which shows an asymptotic significance of 0.001, it can be concluded that there is a significant difference between samples with a 95% confidence level.

Mycofoam Production. Figure 4 shows the incubation results of mycofoam molding. The period of mycelium growth in the mold is 3 to 5 weeks. Once full, mycofoam products are ready to be heated for 8 hours. The heating time was determined after optimization of the heating time of one of mycofoam and met the requirements for the reduced water content of 65%. The characteristics of mycofoam before being heated in each formulation were different as indicated by the appearance of the substrate and mushroom mycelium. P1R on both mushrooms has the smoothest and flattest surface texture due to its 100% sawdust content. After adding bagasse to the P2R, P3R, P4R, and P5R formulations, the roughness level increased. Mycelium density tended to decrease with the addition of bagasse. Besides, the morphology of the mycelium in *P. ostreatus* and *L. edodes* is different. The mycelium of *P. ostreatus* has a cottony texture and a fairly high density, while the mycelium of *L. edodes* has a floccose texture and a fairly low density. Mycofoam made from *L. edodes* also has a brown film.

The difference in mycofoam before being heated was directly proportional to after being heated, where the appearance after being heated was visible because the water content decreased as shown in Figure 5. The texture of mycofoam after heating is very rigid, so it is stronger than before heating. After its appearance was observed, mycofoam was weighed to determine whether its moisture content had been reduced by at least 65% (Table 2). After

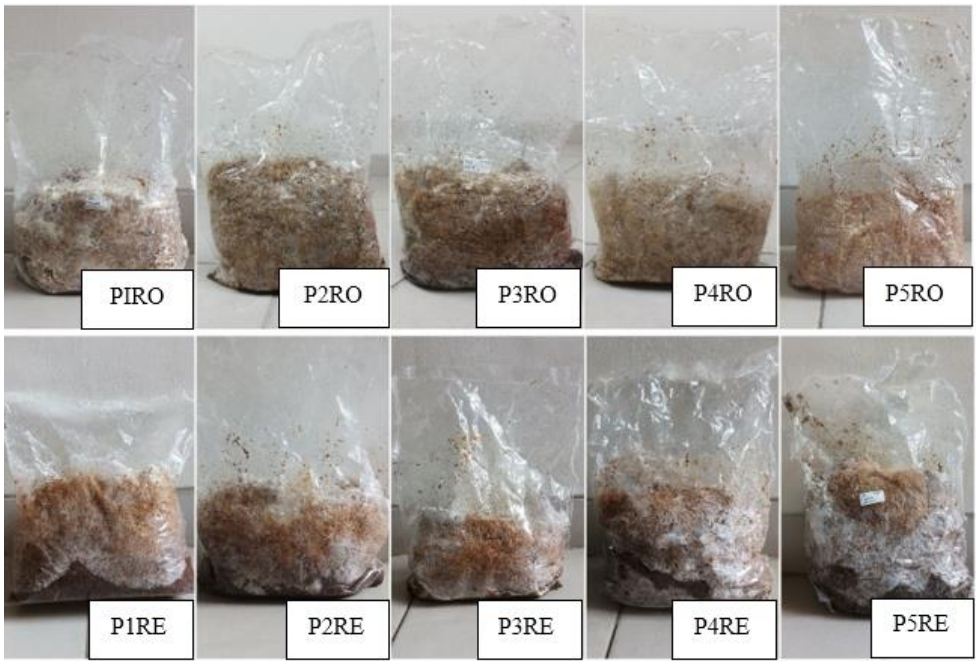

Figure 2.Mycelium growth on growing medium (P1RO-P5RO) *Pleurotus ostreatus*, (P1RE-P5RE) *Lentinula edodes*

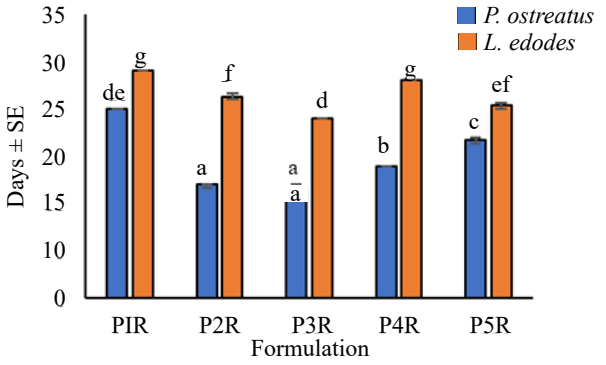

Figure 3. The comparison of the mycelium growth rates of the two mushroom in various formulations

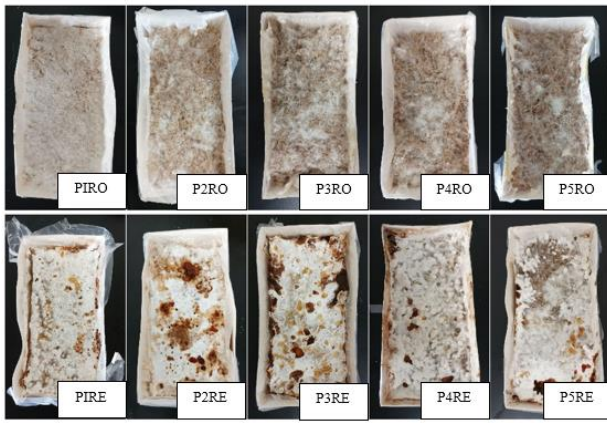

Figure 4. The appearance of mycofoam before being heated (PIRO-P5RO) *Pleurotus ostreatus*, (P1RE-P5RE) *Lentinula edodes*

being weighed, the mycofoam was sorted according to the same dimensions and continued for testing.

Mycofoam Compression and Water Absorption Test. The compressive test was carried out using representatives of each formulation with no repetitions. The results of the compression test can be seen in Figure 6. Based on the results of the compression test, the strongest mycofoam with *P. ostreatus* is P4R formulation, while the weakest is P5R formulation. Meanwhile, the strongest mycofoam with *L. edodes* formulation is P1R, while the weakest is P4R formulation. Compared to the two types of mushrooms, the formulation of P1R *L. edodes* is the strongest with a tolerable force of 8 kgf/cm^2.

Furthermore, water absorption testing was carried out with three repetitions to determine the water resistance of each mycofoam formulation. The results of the water absorption test are presented in Figure 7. Based on the water absorption test result, the highest water absorption of mycofoam using *P. ostreatus* is the P5R formulation, while the lowest absorption is the P4R formulation. However, the highest water absorption of mycofoam using *L. edodes* is the P1R formulation, while the lowest absorption is the P4R formulation. Based on Friedman's non-parametric test with asymptotic significant results of 0.01, the comparison of the water absorption test has a significant difference with a 95% confidence level.

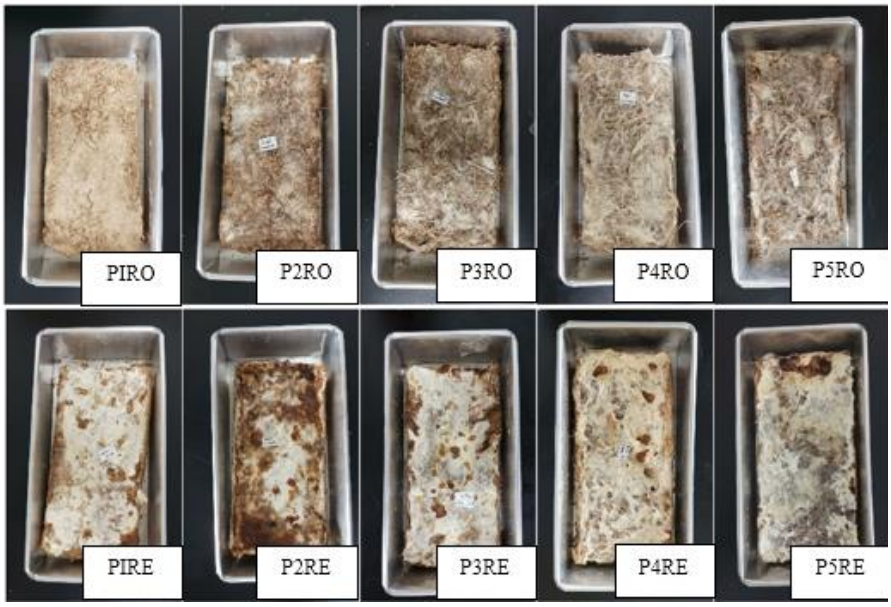

Figure 5. The appearance of mycofoam after being heated (P1RO-P5RO) *Pleurotus ostreatus*, (P1RE-P5RE) *Lentinula edodes*

Table 2. Water loss of mycofoam

Formulation	Mycofoam's water loss (%) ± SD	
	P. ostreatus	*P. ostreatus*
P1R	71.49±4.35	72.12±3.34
P2R	67.89±1.77	70.42±4.46
P3R	72.29±5.03	73.01±1.63
P4R	69.74±3.41	70.60±2.53
P5R	75.73±5.81	69.90±2.24

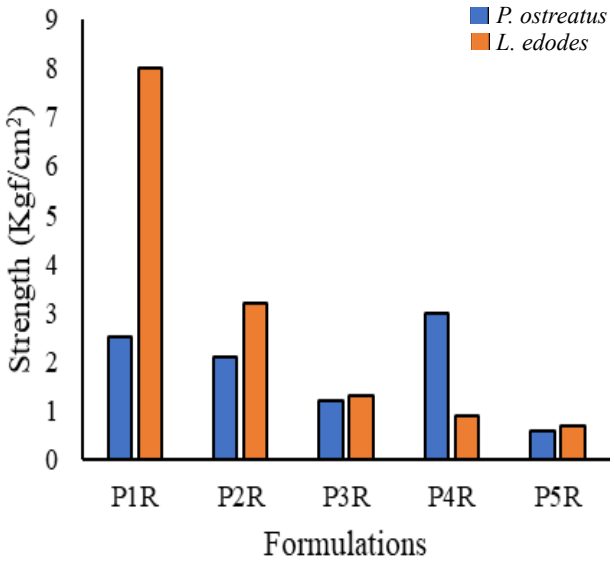

Figure 6. The comparison of mycofoam compression test results

Besides its water absorption, mycofoam is not easy to decompose quickly in water for 24 hours, but its texture starts to soften and brittle.

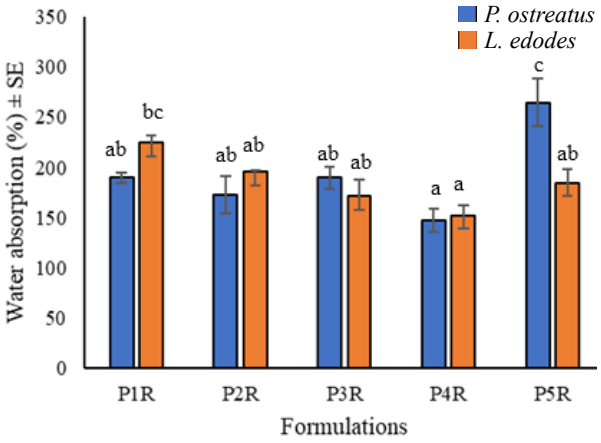

Figure 7. The comparison of mycofoam water absorption

DISCUSSION

The formulations of the growing medium for mycofoam products are detailed in Table 1, which consists of a combination of agricultural waste substrates in the form of Sengon wood (*Albizia chinensis*) sawdust and bagasse (*Saccharum officinarum*). The formulations of the growing medium for these three types of mushrooms were selected based on their high cellulose, hemicellulose, and lignin content to help provide an adequate food source for the growth of mycelium. Sengon wood sawdust contains 36.73% of cellulose, 25.67% of hemicellulose, and 32.93% of lignin (Nurika *et al.* 2017). Meanwhile, bagasse contains 34.5% cellulose, 29% hemicellulose, and

22% lignin (Sidana and Farooq 2014). When these two types of substrates were combined in one formulation, there were differences in the content of cellulose, hemicellulose, and lignin in each formulation. The lignin and cellulose content is higher in the formulation with more sawdust, while hemicellulose is higher in the formulation with more bagasse. These differences in content can influence carbon production, thereby impacting the biomass of the mycelium (Wu *et al.* 2019).

The growth of mycelium becomes the main consideration in the manufacture of mycofoam. This study aligns with research conducted by Permana et *al.* (2009), that both types of mushroom mycelium can grow well on sawdust, bagasse, or their combination substrates. Additionally, *P. ostreatus* demonstrated a quicker colonization and utilization of organic material in the substrate compared to *L. edodes* (Permana *et al.* 2009). The ability of mushroom mycelium to produce lignocellulolytic enzymes plays a major role in breaking down lignin, cellulose, and hemicellulose polymers which are used for mycelium growth. Lignocellulolytics are divided into three groups according to their duties: ligninase to break down lignin, hemicellulase to break down hemicellulose, and cellulase to break down cellulose. Nurfitri *et al.* (2021) reveal that *P. ostreatus* has higher ligninase ability than hemicellulase and cellulase in the vegetative phase (mycelium growth phase). Therefore, mycelium growth is also likely to be denser in formulations with higher lignin content due to the utilization of more nutrients. The ability of *L. edodes* to break down lignin also tends to be better than *P. ostreatus* based on Montoya *et al.* (2015) which used oak sawdust, coconut husks, and soybean oil as substrates. This is directly proportional with the growth of mycelium *P. ostreatus* and *L. edodes* which are denser in formulations with high lignin content.

The effect of chitin content on mycelium also affects mycofoam yield. Chitin provides reinforcement and strength to cell walls of mycelium (Yang *et al.* 2021). Covalently bonded chitin and glucan also play an important role in producing impressive composite architectures (Nawawi *et al.* 2020). Vetter (2007) shows that the chitin content in *P. ostreatus* has an average of 3.78% and 2.8% after demineralization (DM) in the fruit body and stipe, respectively. The chitin content of *L. edodes* in fruit body and stipe has an average of 8.07% DM and 6.55% DM, respectively. This difference in chitin content can affect the strength of the structure in mycofoam (Yang *et al.* 2021).

The results of mycofoam molding showed that the mycelium could bind the substrate well in each formulation. Based on the characteristics after observation, mycofoam has several similarities with EPS, namely light, rigid, and brittle (Adi *et al.* 2020). But the P1R formulation has the most similar appearance because the main substrate was sawdust which tends to have a smoother and flatter surface. This appearance similarity can be the main benchmark for mycofoam as a substitute for polystyrene. After the characteristics were observed, mycofoam was tested to strengthen the data by considering other parameters such as strength and water resistance. The strongest mycofoam in the *P. ostreatus* formulation is P4R, while in the *L. edodes* formulation is P1R, with strengths of 3 Kgf/cm^2 and 8 Kgf/cm, respectively. The formulation of P1R *L. edodes* is the strongest compared to other formulations. The use of sawdust substrate also resulted in higher compressive strength than bagasse as revealed by Joshi *et al.* (2020). This is due to the effect of larger substrate particle size and low mycelium penetration. The higher chitin content in *L. edodes*, the stronger the mycofoam. In the water absorption test, the most water-resistant mycofoam was the P4R formulation in both types of mushrooms, but the *P. ostreatus* formulation tended to be slightly more water-resistant than the *L. edodes* formulation with a water absorption capacity of 147.2% and 152%, respectively. The high water absorption of mycofoam is due to the water absorption factor carried out by the substrate, even though the dried mycelium is hydrophobic (Joshi *et al.* 2020).

These results could be more relevant if polystyrene standards were used in the test. The most common standard for polystyrene compressive test is ASTM (American Standard Testing and Material) D-1621, which is usually the standard for polystyrene for use as building insulation. This standard cannot be used for this research due to the dimensions of the mycofoam sample do not meet the requirements. The most commonly used standard for the water absorption test of polystyrene is ASTM D570. The result of this research was very different from the standard set in ASTM D570 with a water absorption range of 0.01 to 0.30%. This indicates that the use of mycofoam as waterproof polystyrene is very limited and it is supported by Appels *et al.* 2019 and Nava *et al.* 2016. However, in the study conducted by Aquino *et al.* (2022) and Sağlam and Acun Özgünler (2022) shows that the parameters for testing mycofoam have not been well standardized, so the tests carried out in this study have not fully met the original polystyrene standards.

Based on the results of this study, mycofoam has the potential to replace various kinds of objects, such as egg containers, bottle packaging, containers, boards, and other hardware (Figure 8). To increase the function of water resistance, it is necessary to do a coating using biodegradable materials to avoid eliminating the

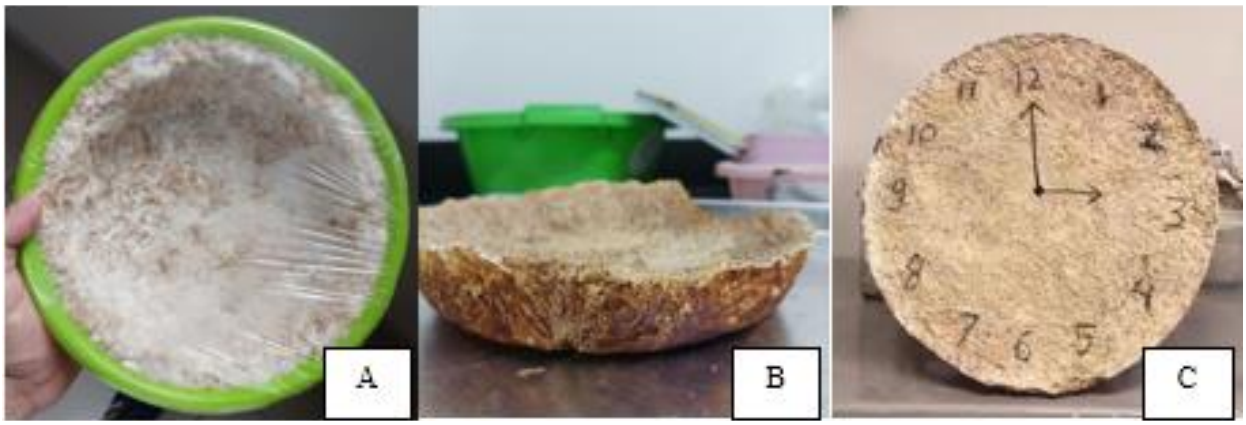

Figure 8. Mycofoam-based innovation (A) plate before heating, (B) plate after heating, (C) wall clock frame

main purpose of this product, namely, environmentally friendly. Based on research conducted by Mokhothu & John (2017), one of the bio-based coating materials that effectively reduces water absorption is polyfurfuryl alcohol (PFA). The development of this waterproof mycofoam can certainly be continued in the future by considering better quality and quantity. Mycofoam must be developed in the long term so that environmental pollution from polystyrene can be resolved properly.

The combination of 100% sawdust substrate and *Lentinula edodes* as the main compositions becomes the most appropriate choice compared to other formulations. This is because the final product of mycofoam from this substrate and mycelium is the closest substitute for polystyrene based on its strength and appearance. The results of the compression test can still be used as a basic reference even though the standard cannot be used. However, the results of water absorption showed that all of these mycofoam formulations are not resistant to water, therefore it is possible to add a bio-based coating for the mycofoam.

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