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A Preliminary Study of Food Waste as a Potential Sustainable Green Substrate for the Oyster Mushroom Cultivation

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The global surge in solid waste, projected to escalate by a staggering 70% annually until 2050, presents a critical environmental and public health challenge. A substantial component of this waste stream comprises food waste originating from the food industry and households, contributing to landfills and associated problems. In response to this issue, this research advocates for the conversion of common food waste items, including coco peat (CP), tea waste (TW), chicken eggshells (ES), and banana skin (BS), into an environmentally sustainable resource for green mushroom cultivation. Traditionally, sawdust has been the primary substrate for mushroom cultivation. In this innovative approach, the food waste materials will partially replace sawdust in mushroom substrate formulation. The mycelium growth was assessed, focusing on mycelium running rate, the duration for complete colonization of the substrate block, the time required for primordia initiation to harvest, average primordia count, fruiting body number, fruiting body dimensions, biological yield, and economic yield. Results indicate significant differences in mycelium growth rates among formulations, with sawdust demonstrating the fastest growth compared to coco peat. However, the inclusion of coco peat in combination with other substrates enhances mycelium growth rates, suggesting the potential for optimizing substrate compositions. CHNS analysis reveals variations in nutrient content among substrates, with formulations featuring higher nitrogen content and C/N ratios correlating with increased mushroom yield where sample S3 with formulation ratio CP: TW: BS: ES (5:2:1:1) afforded the best yield. These findings indicate the importance of substrate composition in influencing mushroom growth and highlight the potential for sustainable waste management practices in mushroom cultivation.

Keywords: Oyster mushroom; food waste; mushroom cultivation; *Pleurotus ostrea*

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By 2050, global waste is projected to rise from two to over three billion tons. Approximately 44% (equivalent to 1.3 billion tons) of these wastes will be categorized as domestic food waste [1]. Typically, food wastes are disposed of in landfills and often incinerated. However, this practice is frequently met with opposition from nearby communities due to concerns about the potential release of toxic compounds like dioxin and polyaromatic hydrocarbons (PAHs), as well as greenhouse gases resulting from the high-temperature (900 – 1000°C) complete oxidative combustion process [2]. The issue of food waste has become a pressing concern for the environment, contributing to the overload of landfills and significant emissions of greenhouse gases [3]. Although approximately 90% of food waste possesses the potential for biodegradability and recycling, the problem arises from its frequent amalgamation with other non-biodegradable waste, hindering effective recycling processes and consequently leading to pollution of land, water, and air [4].Therefore, several studies show fungi like mushrooms present a commendable approach to effectively breaking down recalcitrant macromolecules within food waste into a multitude of smaller fragments [5-7]. This process holds significance for fostering the growth and maturation of mushrooms.

Moreover, food waste surprisingly keeps valuable nutrients suitable for the cultivation of mushrooms. However, it is crucial to note that improper formulation of these waste-derived substrates might impede the

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optimal growth of mushrooms. Hence, a comprehensive investigation becomes imperative to assess the growth performance of mushrooms cultivated from such food waste substrates. Interestingly, the conventional use of sawdust as a substrate for mushroom cultivation lacks circular economic advantages [8]. In this context, channeling the collection and transformation of food waste into a productive avenue like mushroom cultivation could potentially pave the way for a new and sustainable green circular economy, particularly relevant during the transition from the pandemic to the endemic phase.

Pleurotus spp., commonly known as oyster mushrooms, contributes to over 16% of the global mushroom production [9]. Notably, in Malaysia, oyster mushrooms stand out as the predominant cultivated species, constituting around 90% of total mushroom production, with expectations of reaching 65,000 tons by 2030 [10]. Consequently, this heightened mushroom production leads to a proportional increase in the utilization of sawdust, a prevalent substrate for oyster mushrooms, which unfortunately lacks a viable recycling avenue for other waste materials [11]. To address this issue, proactive measures must be taken to redirect food waste away from landfills by embracing a circular economy model, thus transforming it into valuable resources.

Table 1 presents examples of various materials used as a substrate for mushroom species cultivation. Hence, in the selection of food waste materials, this study employed bibliometric analysis (Figure 1) in order to facilitate the identification of the mapped keywords, a manual screening process revealed several keywords with potential relevance. These encompass a range of materials such as wheat straw, rice straw, cotton, tomato, animal feed, diaper, eggshells, banana skin, paddy straw, sawdust, tree leaves, chicken manure, sugarcane bagasse, wheat bran, coffee pulp, crop straw, and peat, all of which could potentially serve as growth substrates for mushrooms. It's important to highlight that, to our knowledge, no existing studies have explored the combination of coco peat, banana skin, chicken eggshells, and tea waste as a growth substrate. This unique approach undoubtedly underscores the novelty and originality of the proposed research.

Primarily, the successful cultivation of oyster mushrooms hinges on the availability of cellulose, lignin, nitrate, phosphate, and potassium. As depicted in Figure 2, a proposed environmentally conscious approach involves repurposing food waste for use as a media substrate in mushroom cultivation. The requisite cellulose and lignin components can be sourced from discarded coco peat (CP), while essential minerals like calcium (Ca), phosphate (P), and a nitrogen (N) source can be derived from eggshells (ES), banana skins (BS), and tea waste (TW), respectively [18]. Five (5) samples were formulated as media substrates as listed in Table 2.

Table 1. Examples of various materials used as a substrate for mushrooms.

Growing media substrate	Mushroom species	References
Corncobs, finger millet straw, and bamboo waste	*Pleurotas astreatus*	[12]
Reeds	*Auricularia auricula-Judae*	[13]
Straw rice, and powder saws	*Pleurotas astreatus*	[14]
Corncobs and sawdust waste	*Pleurotas astreatus*	[15]
Mulberry waste	*Pleurotus ostreatus*	[16]
Sawdust and bran rice waste	*Pleurotus ostreatus*	[17]
paddy straw, maize cob, sugarcane bagasse, and sawdust waste	*Pleurotus ostreatus*	[18]
paddy straw, sugarcane bagasse and banana leaves waste	*Pleurotus ostreatus*	[19]

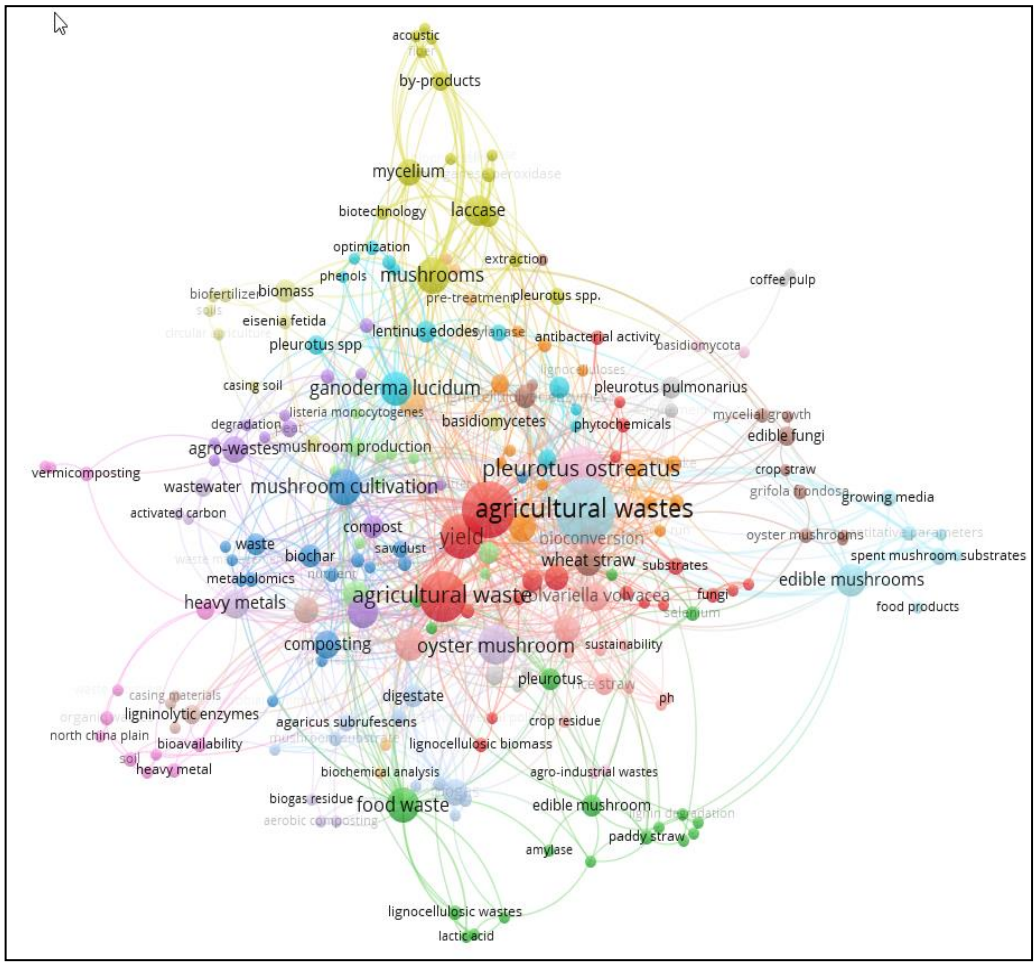

Figure 1. Bibliometric analysis to choose the food waste material as media substrate.

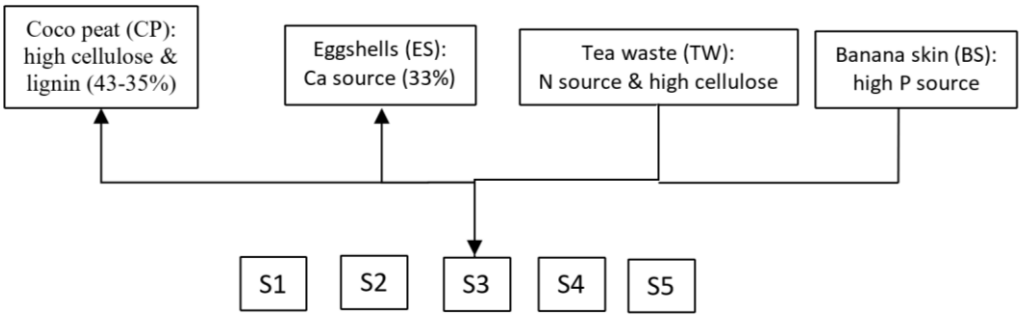

Figure 2. Media substrate for mushroom cultivation.

Thus, the primary objectives of this study are twofold. Firstly, the aim is to develop a novel formulation utilizing a combination of food waste components, including coco peat, banana skin, chicken eggshells, and tea waste, to create an optimized growth substrate for oyster mushrooms. This endeavor seeks to harness the potential of these discarded materials, repurposing them into a substrate that fosters the growth and development of oyster mushrooms. Additionally, a comprehensive analysis of the physicochemical properties of the resulting mushroom substrate blocks was undertaken. Secondly, this study intends to conduct an in-depth investigation into the growth performance of oyster mushrooms when cultivated on the formulated food waste-based substrate. This examination has provided crucial insights into the compatibility and effectiveness of the food waste-derived substrate for oyster mushroom

cultivation, shedding light on the potential viability of such a sustainable and circular approach to both waste management and mushroom production.

MATERIALS AND METHODOLOGY

Mushroom Source

Grey oyster mushrooms in their mycelium-enriched spawning state were acquired by Uwais Cendawan Agrotech Enterprise, Jalan Pantai, Seremban. The mushroom spawn was preserved at 4 °C prior to use.

Food Waste Collection

As the main aim of the study is to valorize food wastes as mushroom cultivation substrate, therefore, food waste such as banana skin was collected from a banana fried stall, chicken eggshells and used tea from the stall at Senawang town and cocopeat was provided by HTC Coconuts Sdn. Bhd. The banana skin and used tea wastes were individually subjected to drying in an oven at 70 °C for 24 hours while the eggshell was dried at room temperature. Then, the dried food waste was grounded using a mechanical blender and stored in an air-tight container for the subsequent formulation of the mushroom substrate.

Preparation of Media Substrate

This study utilized an adapted method based on [20]. As this study was a preliminary project, hence, the experiment was conducted using a test tube. The mushroom growth substrate was prepared by varying the weight ratios of food waste as shown in Table 2. The pH of the growth substrate was adjusted to pH 6 ± 0.5 by adding an aliquot amount of gypsum. Sawdust, cocopeat, and food waste were thoroughly moistened and loaded followed by formulation ratio in a small tray. The moisture content of the substrate was kept constant at 75 ± 5%. About 30g of substrate sample was packed in each test tube and each substrate sample was prepared in triplicate.

Next, all the samples were autoclaved at 121 °C for 120 min and kept in a clean room before the spawning procedure. Next, the sterilizing process was repeated triplicates to ensure that any contaminants or undesired microorganisms in the substrate were entirely removed. The sterilized substrate test tube was then removed from the autoclave and allowed to cool overnight on the clean bench before the inoculation process.

The mushroom cultivation process started with the addition of a spatula (approximately 10 beans) of mushroom culture into each growth substrate test tube (Figure 3). As a precautious step, the inoculation process were carried out aseptically in a sterilized environment in the clean bench with the spatula were heated for a minute and soak in alcohol 70%. This precaution step was taken to reduce the possibility of contamination from both outsides and within the inoculation workplace into the substrate test tube. The clean bench also was treated by UV light for 10 minutes before inoculation process.

Table 2. Formulation of growth substrate in different weight ratios of cocopeat to food waste.

Substrate sample	Formulation
S1	Commercial mushroom cultivation substrate - 100% sawdust (control)
S2	CP
S3	CP: TW: BS: ES (5:2:1:1)
S4	CP: TW: BS: ES (6:3:1:1)
S5	CP: TW: BS: ES (7:4:1:1)

Notes: CP - cocopeat, TW – tea waste, BS – banana skin, ES – chicken eggshell.

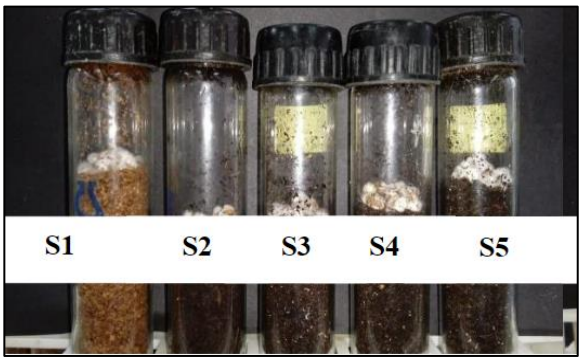

Figure 3. Mushroom cultivation.

Spawning and Culturing Conditions of Mushroom

The test tube is positioned vertically within the test tube rack to expedite the mycelium spawning process. This phase, which spans 15 to 20 days from the initiation of growth and development, is influenced by factors such as temperature, humidity, and light conditions. Throughout the spawning phase, a temperature range of 28-35 °C and humidity levels between 75-85% are upheld [21]. To maintain suitable humidity levels, routine applications of plain water are sprayed onto the mushroom house's floor, walls, and test tube. During this stage, light intensity remains relatively low, and the substrate test tube is shielded with a sun shade.

The test tube rack serves as a surrogate mushroom house and is strategically positioned to minimize exposure to dust and pests. Stringent measures are implemented to optimize the cleanliness and sanitation of the mushroom test tube rack's surroundings on a daily basis, thereby mitigating the risk of contamination.

Growth and Yield of Mushroom

After the mycelium had colonized the substrate completely (Figure 4a), the cap of the mushroom test tube was unsealed, creating a conducive environment for the emergence of pinheads. To gather information about the pinheads, primordia, and subsequent flushing stages, it was necessary to extract the substrate containing oyster mushroom mycelium from the test tube. The mycelium-infused substrate was deliberately extracted from the test tube to induce the initiation of pinning. To facilitate this, the substrate was enclosed with plastic wrap as depicted in Figure 4b. This wrapping was used as a means to observe the progression from pinning to the formation of primordia. Over a span of 4 to 7 days from the time the cap was opened, pinheads called primordia gradually emerged from the substrate, marking the onset of the pinning phase. Subsequently, the primordia underwent growth, developing into distinct, rounded structures that formed the noticeable fruiting bodies.

Multiple criteria were evaluated as part of the study of mushroom growing performance. These variables included the mycelium running rate (MRR), the number of days needed to completely colonise the substrate, the time needed to go from stimulation to primordia initiation, and the number of days from primordia initiation to harvesting. The analysis also included the average number of primordia, average numbers of fruiting bodies and effective fruiting bodies, average physical observation on fruiting body sizes (cm) such as size and thickness of pileus, length and diameter stipe, average biological yields (g), and average economic yields (g).

Weight Reduction of Media Substrate

As part of its growth phase, the mushroom assimilates and breaks down organic components from both cocopeats and food waste, as seen by the weight loss. The weight reduction of the substrate block also could serve as an effective indication on how the mushroom utilizes the food waste and lead to a significant decrease in the landfilling of food wastes. The media substrate were weighed after the mushrooms were harvested in order to determine how much weight had been lost in each substrate formulation. The specified formula was used to calculate this amount as stated in Equation 1.

Where W_i was the initial weight of substrate and W_f was the final weight of substrate.

$$\text{Percentage weight reduction} = \left(\frac{W_i - W_f}{W_i} \times 100\right) \qquad \text{(Equation 1)}$$

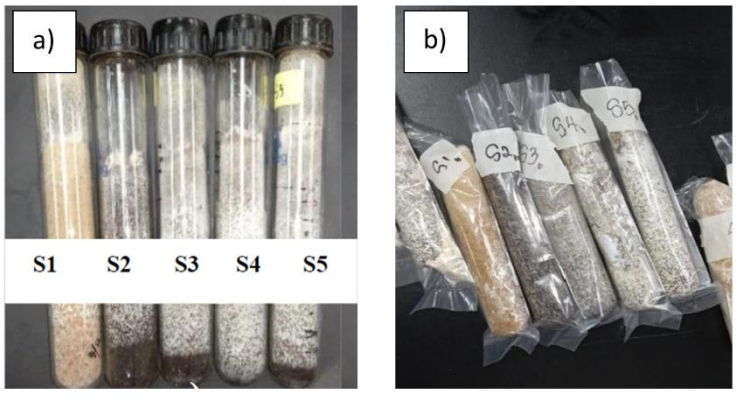

Figure 4. a) Mycelium fully growth filled the media substrate b) Substrate transferred into plastic wrap.

Table 3. Mycelium growth in the various substrate formulation.

Samples	Formulation	Average maximum mycelium length (cm)	Days required to complete mycelium run	Mycelium running rate (cm/d)
S1	Saw dust	10.33_a	14	0.7381
S2	Cocopeat	9.50_a	20	0.4750
S3	CP:TW:BS:ES	9.97_a	19	0.5246
S4	CP:TW:BS:ES	9.97_a	15	0.6733
S5	CP:TW:BS:ES	10.07_a	18	0.5593

Mean with the same subscript in the column are not significantly different at $p<0.05$

Characterization Methods

CHNS Analysis of Media Substrate

The nutritional composition of the substrate altered with food waste plays a pivotal role in facilitating optimal mushroom growth. Therefore, conducting a CHNS analysis becomes imperative as it can unveil the ideal Carbon: Nitrogen ratio, particularly when various weight ratios of food waste constituents are being examined. Previous research indicates that an excessive presence of Nitrogen (N) can also be counterproductive for mushroom growth, potentially leading to unwanted substrate contaminations. In this study, an assessment has been conducted on the constituent elements of media substrate and nutrient supplements, encompassing Carbon, hydrogen, nitrogen and sulfur contents as well as the functional groups using FlashSmart CHNS. Media substrates were treatment by ground into fine powdered form < 2mm.

Data Analysis

Triplicate samples were utilized for all analyses, and the resulting mean values were prominently featured in the results section to enhance the data interpretation's significance. Data analysis was conducted employing the Statistical Package for the Social Sciences (SPSS) version 26. Furthermore, Pearson's correlation analysis was executed to explore relationships between media substrates. To facilitate a comprehensive understanding, a one-way ANOVA analysis was carried out to compare the media substrate, followed by the post hoc Tukey test for assessing mean media substrate distinctions. Statistical significance was recognized at a level of $p < 0.05$. For graphical representation, Microsoft Excel 365 served as the chosen tool.

RESULTS AND DISCUSSION

Table 3 showed the mycelium growth in the sample. In overall there is no significant difference in the mycelium growth in all sample. However, the S1 sample produced faster growth compared to others and was completely permeated on day 14. In contrast, mycelium growth was slowest in the S2 sample after 20 days. Different formulations lead to a different speed of mycelium growth. This is to be expected as the composition of the substrate in the samples is different and could influence the ability of the enzymatic reaction. Sample S2 was formulated with cocoa peat, which has previously been reported to induce slower mycelial growth compared to sawdust [21]. The study indicated that mycelial growth is hindered by higher density in 100% cocoa peat composition [22]. When the cocopeat was mixed with other types of substrates, a better mixture composition was produced, which has lower density and allows better mycelial growth. However, there was no significant difference between the samples in terms of the length of mycelium growth as determined by statistical analysis. The maximum length of the mycelium reached after complete permeation was the same for all samples.

Table 4 presented the mushroom yield of various substrates. This study is carried out in triplicate and the average data is shown in Table 4. Since this study used a small sample for the cultivation of *Pleurotus* sp., it is expected that the yield will be much lower compared to similar studies with a larger sample. In addition, this study was designed to determine the potential of different agricultural wastes as a mushroom substrate without additional nutrients. In all samples there is a significant different in the weight of mushroom harvested. Sample S3 provided the best yield with a biological efficiency of 5.6%. Other samples such as S1, which consisted of sawdust, and S3, which was mixed with tea waste, had a lower biological efficiency of 3.55 and 3.82 % respectively. Enrichment of mushroom substrate with Ca is a common strategy to increase yield and also for pH balance and control of contamination [23]. Various inedible Ca sources have been reported to be used, such as agricultural lime, starfish powder, eggshells, oyster shells, etc., which contain $CaCO_3$ as a major component [24-25]. In samples S3 to S5, eggshell powder was added as part of the formulation.

Table 4. Mushroom growth performance of various substrate formulation.

Samples	Formulation	Fruiting Bodies Development			Biological Weight (g)	Biological Efficiency (%)
		Numbers of Pin Head	Numbers of Primordia	Flushing		
S1	Saw dust	11.67_a	1.33_a	0.67	0.3553_a	3.55
S2	Cocopeat	66.67_b	17.33_b	1	0.17_b	1.7
S3	Cocopeat + tea waste+ banana skin +eggshells	20.33_c	13.67_c	1	0.5651_c	5.6
S4	Cocopeat + tea waste+ banana skin +eggshells	96.67_d	24.67_c	1	0.3822_a	3.82
S5	Cocopeat + tea waste+ banana skin +eggshells	165.33_e	30_e	2	0.1657_d	1.65

Mean with the same subscript in the column are not significanly different at $p<0.05$

However, the highest amount of eggshells added was in S3, followed by S4 and S5. The higher concentration of eggshells was found to contribute to higher mushroom yield. This indicates the importance of Ca as a supplement to increase yield. Julian et al (2018) reported that the addition of 6% eggshell powder in the substrate improved yield by up to 35% [26]. A better yield was recorded in a sample with a higher amount of dried banana peel added at a similar concentration as eggshell powder. A study conducted by [27] found that a 5% concentration of kepok banana peel powder added to the mushroom strain gave a higher yield compared to the control, while the tea waste component did not contribute much to the yield. It was also observed that the number of pinheads and primordia developed did not correlate with the development of fruiting bodies. This was observed as the growth from pinhead to primordia and to mature fruiting bodies did not progress well as a majority of the pinheads did not grow into mature fruiting bodies. This could be due to the size of the mushroom compost, which is smaller compared to the commercial mushroom log, which weighs around 600-800g.

Table 5 displays the percentages obtained through CHNS analysis. Sulphur is not detected in any of the samples, while Carbon consistently records the highest percentage across all formulations. This dominance is attributed to the presence of carbon-based materials like sawdust and cocopeat in all substrates. The Nitrogen percentage is notably low in S1 and S2, indicating the absence of food wastes such as banana skin and tea wastes. The introduction of these wastes in formulations S3-S5 has led to an increase in the Nitrogen percentage. Nitrogen is crucial for mushroom growth, but an excess may lead to rapid contaminations. In this study, a comparison among S3-S5 reveals a higher C/N ratio, which is expected to enhance the growth mechanism of the mushrooms [28].

As noted in previous research, anaerobic digestate has demonstrated the ability to enhance fruiting body development when applied to lignocellulosic waste with a high C/N ratio. Furthermore, it is recommended that the nitrogen concentration of the substrate falls between 1.84 and 2.08. Oyster mushrooms, not requiring high nitrogen concentrations, especially during the initial substrate colonization, can benefit from nitrogen addition for enhanced fruiting bodies. However, excessive nitrogen can suppress the fruiting phase of the mushroom [29].

Table 5. CHNS Analysis of mushroom substrates with different formulations.

Substrates	C%	H%	N%	S%	C/N
S1	45.737	5.758	0.047	0	973.128
S2	43.147	4.885	0.977	0	44.163
S3	41.901	4.786	2.096	0	19.991
S4	41.113	4.860	2.329	0	17.652
S5	40.098	4.575	2.168	0	18.495

Biological Yield vs Economical Yield

Biological yield refers to the total weight of the entire cluster of fruiting bodies, while economic yield encompasses the weight of the fruiting bodies excluding the lower hard portion of the mushroom. The harvested mushrooms' biological and economic yields are detailed in Table 6. Notably, the growth substrate S3 demonstrated superior mushroom yields, both biologically and economically, compared to other substrate formulations. This enhanced yield is attributed to the incorporation of food wastes such as banana skin, eggshells, and tea powder in the mushroom substrates. Additionally, these food wastes contributed to a high C/N ratio, a critical factor in mycelium colonization and fruiting body formation during mushroom cultivation. As highlighted in Table 5, among the formulations S3-S5, S3 exhibited a higher C/N ratio, likely the primary factor behind its elevated biological and economic yields [29].

Weight Reduction

Examining weight reduction is a crucial aspect to gauge the effectiveness of the mushroom substrate formulation in repurposing food wastes and preventing their disposal in landfills. Figure 5 illustrates the percentage of weight reduction in relation to the substrates after harvesting the mushrooms. Interestingly, S2, composed entirely of cocopeat, demonstrated superior waste utilization with an 81.43% reduction. However, this substantial waste reduction doesn't align with the mushroom yield reported in Table 6. This discrepancy suggests that 100% cocopeat may have degraded significantly without significantly contributing to the overall growth of the mushroom substrates.

In contrast, the comparison between S1 (control) and formulations S3-S5 revealed that S3 exhibited the highest weight reduction, aligning well with the findings in Table 6. The mechanism driving waste utilization and reduction is likely associated with cellulose-degrading activity. This phenomenon has been observed by other research groups as well [30-32].

Table 6. Biological yield vs Economical yield of the harvested mushrooms.

Substrate	Biological yield (g)	Economical yield (g)
S1	0.3553	0.0890
S2	0.1700	0.0898
S3	0.5651	0.4019
S4	0.3822	0.1962
S5	0.1657	0.1081

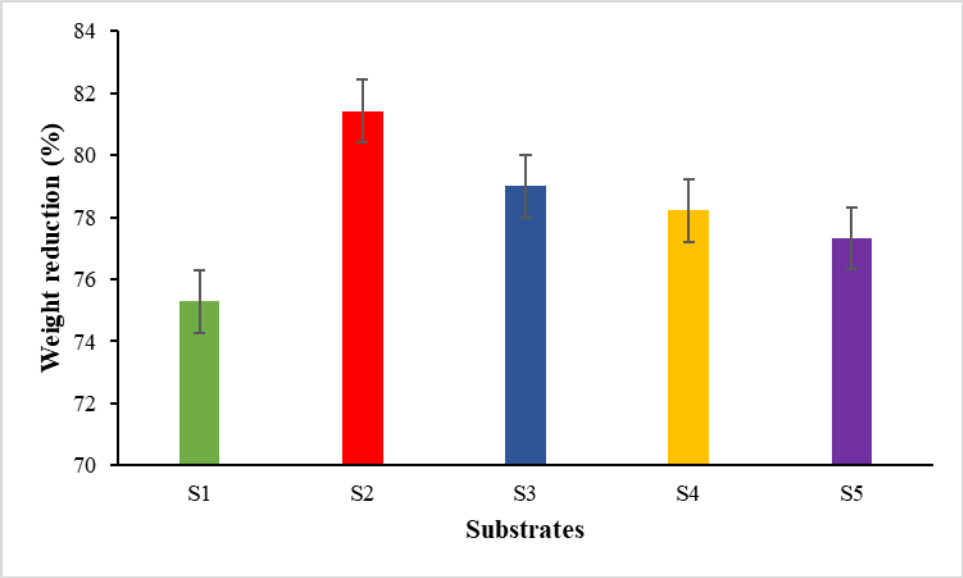

Figure 5. Weight reduction of substrate blocks after the mushroom harvest (n=3).

CONCLUSION

In summary, the feasibility of using food waste as a sustainable green substrate for oyster mushroom cultivation has been investigated in this study. According to the findings, sample S3 afforded the best yield with a biological efficiency of 5.6% which was supported by CHNS analysis that nitrogen percentage is notably low in S1 and S2, indicating the absence of food wastes such as banana skin and tea wastes. Moreover, S3 exhibited the highest weight reduction indicating the nutrients successfully utilized for the development process. These results point to a viable direction for addressing the requirement for sustainable agricultural practices as well as environmental issues pertaining to the disposal of food waste. In addition to providing an environmentally friendly way to manage waste, using food waste as a substrate for oyster mushroom cultivation also presents a financially feasible choice for community-based and small-scale mushroom cultivation initiatives. The results of this study have applications outside of the lab, suggesting a feasible and scalable method for repurposing food waste in the framework of sustainable agriculture.

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