

Pleurotus ostreatus Cultivation: Physico-Chemical Characteristics of a Robust Pre-Blocks Oyster Mushroom Substrate with Absorptive Starch Binders

(Pengkulturan *Pleurotus ostreatus*: Pencirian Fiziko-Kimia Substrat Pra-Blok Cendawan Tiram Mampan dengan Penjerapan Kanji Pengikat)

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ABSTRACT

An alternative way of preparing a newly robust and low fragility of the Pleurotus ostreatus (oyster mushroom) cultivation media is needed as this major problem occurred during harvesting and recyclability. Thus, the cultivation of P. ostreatus mushroom in the form of robust blocks bound together with starch using different tapioca flour binder concentration of 5%, 10% and 25% (w/v) was evaluated for cultivation method development. How mushroom block binder material affects the biological efficiency (BE) and its vegetative growth on newly robust media were identified. The optimized composition of the conventional mushroom substrate has been prepared as a benchmark with the ratio of 100: 10: 1 to residual sawdust, agricultural rice bran and agricultural lime prior to binder mixing. Mushroom BE from bag cultivation methods had been used as a control, which produced lower BE (13%, w/w) than the blocks with a binder (ranging from 18 to 29%, w/w) ($p < 0.05$). The mushroom block with a concentration of 25% (w/v) tapioca flour was found to have a higher bulk density with an average value of 0.87 g/cm^3 as compared to the mushroom blocks of 10% (w/v) and 5% (w/v) tapioca flour ($p < 0.05$). The highest water absorption was obtained in the block with 5% (w/v) tapioca flour with a value of 300.18% (w/w) on a dry basis. In terms of compressive strength, the substrate of the mushroom block from 25% (w/v) tapioca flour had the highest value with 0.022 N/mm^2 . The pH value of the substrate before seeding was between 8 and 9 whereas post-seeding value was measured at 4 to 6. The physico-chemical analysis of density, water absorption, compressive strength test, pH and colour values exhibited a good and ideal micro-environment growth of the mushroom. Some nutritional deficiencies in the pre-blocks were observed through imperatively acceptable as compared to the compressed substrate in polypropylene bags (PP). The pre-block formulations with 5% (w/v) and 25% (w/v) tapioca flour conceive good potential and potency in producing high BE of P. ostreatus without compromising its nutritional health benefits.

Keywords: Biological efficiency; oyster mushroom; Pleurotus ostreatus; pre-block substrate; tapioca flour

ABSTRAK

Satu kaedah alternatif yang tahan lasak dan ampuh bagi penghasilan media penanaman Pleurotus ostreatus (cendawan tiram) amat diperlukan memandangkan terdapat beberapa masalah yang berlaku semasa proses penuaian dan kitar semula. Justeru, pembangunan kaedah baru penanaman cendawan P. ostreatus dalam bentuk blok yang tahan lasak, diikat bersama kanji menggunakan pengikat tepung ubi kayu dengan peratus berbeza iaitu 5%, 10% dan 25% (w/v) telah dilakukan. Keupayaan dan bagaimana bahan pengikat blok cendawan mempengaruhi kecekapan biologi (BE) serta pertumbuhan vegetatif pada media baru ini dikenal pasti. Komposisi substrat cendawan konvensional yang dioptimumkan dengan nisbah 100: 10: 1 untuk habuk papan, sekam padi dan kapur daripada pertanian telah disediakan sebagai penanda aras sebelum bahan pengikat dicampur. Cendawan BE daripada kaedah penanaman menggunakan beg yang telah dipilih sebagai kawalan didapati menghasilkan BE yang lebih rendah (13%, w/w) daripada blok dengan pengikat (julat antara 18 dan 29%, w/w) ($p < 0.05$). Blok cendawan dengan kepekatan 25% (w/v) tepung ubi kayu didapati mempunyai ketumpatan pukal dengan nilai purata sebanyak 0.87 g/cm^3 berbanding dengan blok cendawan pada 10% (w/v) dan 5% (w/v) tepung ubi kayu ($p < 0.05$). Penyerapan air tertinggi didapati pada blok dengan 5% (w/v) tepung ubi kayu dengan nilai 300.18% (w/w) berat kering. Dari segi kekuatan mampatan pula, substrak blok cendawan daripada 25%

(w/v) tepung ubi kayu mempunyai nilai tertinggi pada 0.022 N/mm². Nilai pH bagi substrat sebelum penyemaian adalah antara 8 dan 9 manakala nilai pH pasca (selepas) penyemaian didapati berada pada 4 hingga 6. Analisis fizikokimia bagi ketumpatan, penyerapan air, ujian kekuatan kemampatan, nilai pH dan warna menunjukkan mikro-persekitaran pertumbuhan cendawan yang baik dan ideal. Walaupun terdapat beberapa kekurangan dari aspek pemakanan pada pra-blok, ia boleh diterima dan lebih baik daripada substrat yang dimampatkan dalam beg polipropilena (PP). Formulasi pra-blok menggunakan 5% (w/v) dan 25% (w/v) tepung uji kayu berpotensi baik dan ampuh dalam menghasilkan nilai BE yang tinggi bagi *P. ostreatus* tanpa menjejaskan nilai pemakanan dan manfaat kesihatannya.

Kata kunci: Cendawan tiram; kecekapan biologi; *Pleurotus ostreatus*; substrat pra-blok; tepung ubi kayu

INTRODUCTION

Mushroom is a favourable food due to its texture features when bitten and also good in savoury flavour. The mushrooms typically have broad, thin, oyster- or fan-shaped caps and are white, grey, or tan, with gills lining the underside (Dias 2010). In fact, mushrooms receive overwhelming responses from food and pharmaceutical researchers because of the bioactive composition in them (Mariga et al. 2014; Saidu 2013). The bio-molecules found in mushrooms including phenolic compounds, resin structures, steroids and polysaccharides have various biological activities (Shang et al. 2015). For example, the well-known oyster mushrooms such as *P. ostreatus*, *P. cystidiosus*, *P. cornucopiae*, and *P. pulmonarius* are considered the best option for cultivation as they produce high yield, easy to handle and being the best nutritious delicacies in Asia as they are full of micronutrients and vitamins (Dias 2010). Oyster mushrooms are saprophytes that decompose agricultural by-products because they have the ability to use cellulose, hemicellulose and lignin as carbon sources (Dias 2010; Sofi et al. 2014). Therefore, this ability enables the mushrooms to be grown in various types of plant residues. However, the substrate is a major component in mushroom cultivation where several considerations must be taken into account in order to achieve optimal mushroom cultivation. Thus, a few guidelines need to be met and carried out which are as follows: the substrate must be suitable for growth and flowering of the mushrooms; substrate is locally available in sustainable quantities and low in terms of cost; and the climate must be suitable for growth and mushroom breeding (Tisdale et al. 2006). Through this research, the media of mushroom cultivation was provided with substrate mixture consisting of saw dust, rice bran, agricultural lime and water mixed together with a tapioca binder to form a stable and robust scaffold/block. The conventional method of cultivation relies heavily on the polypropylene bags (PP), and it is considered fragile

and ruptures easily. For that reason, a suitable binder material is needed to overcome the problem. The binder is usually composed of fibrous organic matter such as starch, and it also would perhaps be the substitute carbon sources for the growth. The method of *P. ostreatus* mushroom media preparation was modified from the conventional mushroom planting method as a new binder was introduced. Thus, factors for the effectiveness of mushroom growth in the presence of a binder and mycelium were evaluated. The effect on the nutritional substrate/media content, block physico-chemical properties and the mushroom productivity were also assessed.

MATERIALS AND METHODS

MATERIALS

The substrates used were residual sawdust, agricultural rice bran, agricultural lime and fungus seeds (grey oyster mushroom: *P. ostreatus* Strain HK-35 (Sylvan, USA)). All the substances (for the substrate preparation and oyster mushroom strain) were obtained from Nas Agro Farm, Sepang, Selangor, Malaysia (GPS coordinates: 2.9423378, 101.7913518). The substrate and seed preparation process were carried out at the Food Pilot Plant, Faculty of Science and Technology (FST), UKM Bangi under control and sterilized environment. The commercially available tapioca flour (500 g) was purchased from Tesco (M) Sdn. Bhd. supermarket (Kapal ABC Brand Tapioca Starch; ISO 9001: 2008).

MUSHROOM PP SUBSTRATES

Mushroom PP substrate as a control was prepared with the ratio of 100: 10: 1 for residual saw dust taken from rubber tree (*Hevea brasiliensis*), rice bran (*Oryza sativa*; strain variety MR232) and lime (*Citrus aurantiifolia*) which was mixed with 100 mL water. The mixture was agitated using vibrator then manually compressed by hand

in polypropylene bags (PP) with the dimension of 5 cm (height) × 10 cm (width) prior to substrate sterilization process (Saidu et al. 2013).

MUSHROOM PRE-BLOCK SUBSTRATES WITH TAPIOCA BINDER

Pre-block mushroom substrate was prepared with the ratio of 100: 10: 1 for sawdust, rice bran and lime as in the control preparation likewise. Then, binders with concentrations 5% (w/v), 10% (w/v) and 25% (w/v) of tapioca flour (TF) were prepared. The binder then was dissolved into 100 mL water and made into glue and well-mixed with aforementioned substrates. The mixture was inserted and manually compressed by hand in cylindrical moulds measuring 10 cm (height) × 6 cm (width) prior to substrate sterilization process (Saidu et al. 2013). The pre-block substrates were classified and coded into two control PP blocks (negative (-ve) and positive (+ve)) and six different TF concentrations of pre-blocks prior to physico-chemical analysis. The classification are as follows: MSM (+ve control): mushroom substrate with mycelium; MSWM (-ve control): mushroom substrate without mycelium; S5%M: substrate 5% TF with mycelium; S5%WM: substrate 5% TF without mycelium; S10%M: substrate 10% TF with mycelium; S10%WM: substrate 10% TF without mycelium; S25%M = substrate 25% TF with mycelium; and S25%WM: substrate 25% TF without mycelium.

STERILIZATION AND SPAWNING

The mushroom control substrate and mushroom block substrate were sterilized for 4 h in a steam container at 121 °C. After the sterilization process, all the substrates were reinvigorated and cooled for 2 h. The surrounding area and the seed picking were sterilized with 70% (v/v) ethanol to reduce and prevent the external fungi from contaminating the substrate. The prepared substrate was inoculated with an amount of approximately 2 g of mushroom spawn under aerobic fermentation. The spawn mixing and substrate block formation were completed by mechanical vibration. The bag was closed and kept in a clean place under aerial cooling to 25±2 °C for the purpose of the growth of mycelium for 2 months (relative humidity (RH): 80-90%). Vegetative growth was monitored visually every 3 days. The physico-chemical analysis of all pre-block substrates either with spawning mycelium or not were carried out after 2 months of the mushroom growth. The biological efficiency (BE) % was determined using the ratio of the total weight of the fresh mushrooms to the absolute dry weight of the substrates

(Saidu et al. 2013). The average weight of each substrate block was 101±2 g.

PRE-BLOCK SUBSTRATE ANALYSES

The bulk density was determined by dividing the weight of the scaffolding mushroom block with the volume of cylindrical block dimension (1) (Zubairi et al. 2015). The weight of each block concentration was recorded where reading was taken three times for the weight for each type of block concentration. Then, the cylindrical block dimension volume (V) was calculated using the formula $\Pi r^2 h$ (cm³) where: Π (Pi value) = 3.142; r = block radius (estimated) and h = block height.

$$\text{Block bulk density (g/cm}^3\text{)} = \frac{\text{weight of the block (g)}}{\text{volume dimension of block (cm}^3\text{)}} \quad (1)$$

The pH of the block substrate was measured by weighing approximately 1 g from each of the substrate before inoculation and soaked in 10 mL of distilled water (50 mL vial). The substrates were allowed to be soaked for 10 min whilst stirring (Vortexer V-32 Labforce: 400 rpm) prior to pH measurement (Hannan electronic pH meter, Japan). The mixture was then filtered using muslin cloth to remove substrate remnants and the pH were recorded directly from the water extract in triplicates ($n = 3$) (Fazil et al. 2018).

The water absorption capacity was determined by conducting the water absorption tests based on the standard protocol of IS 3495 (Part 2): 1992 (Nagarajan et al. 2014). Three samples (pre-block substrates before being immersed into water) per succession level were weighed, and the weight was recorded and expressed as M1. The block substrates were then immersed in water for 24 h and later removed prior to weighing to determine the weight of the substrates after being immersed into water (M2). Equation (2) expressed the block water absorption capacity after 24 h of immersion in water where the calculations were expressed as dry basis moisture (Che Johari et al. 2017).

$$\text{Water absorption capacity (\%)} = \frac{M2 (g) - M1 (g)}{M1 (g)} \times 100 \quad (2)$$

Mushroom substrate colour before and after the resulting seeding was determined using the Chroma Meter (Model CR-400, Minolta, Japan). The parameters were determined by L* (L* = 0 [black] and L* = 100 [white], a* (-a* = [green] and +a* = [reddish]). The values of those parameters were determined in three random places in each substrate of the propagated fungi ($n = 3$) (Fadzilah et al. 2020).

MECHANICAL TESTING

Compression strength test on block samples was carried out in accordance with the American Standard Test Method (ASTM) International standards in accordance with D 1037-99 standard test methods standard for assessing the properties of wood fibre and particle panel materials. The tool used for the experiments was Universal Testing Machine, Instron Model 5567 (Norwood, MA USA). The pre-block substrates with a dimension of 10 cm (height) × 6 cm (width) were placed between supporters and flat steel on it. Then, the machine used a uniform compression load of 10 kN until the specimen failed. The maximum compressive strength applied to the specimen was set at 80% of failure. The maximum load was recorded for each specimen test. Compressive strengths were calculated as loads per unit area as shown in (3) (Aizad et al. 2017).

$$\text{Compression strength (in N/mm}^2\text{)} = \frac{\text{maximum load at failure}}{\text{contact area}} \quad (3)$$

NUTRITIONAL ANALYSIS ON MUSHROOM PRE-BLOCK SUBSTRATES

Mushroom substrate analysis for protein (remaining), fat and crude fibre content was carried out according to Association of Official Analytical Chemist (AOAC 2012) standard protocols which further details of these analysis are available elsewhere (Ali et al. 2009; Shang et al. 2015).

STATISTICAL ANALYSIS

The results of the statistical analysis were in the form of $\text{min} \pm \text{standard deviation (SD)}$. The difference between the mean values was analysed using the SPSS (Statistical Packages for Social Science) version 24.0 which involved Analysis of Variance (ANOVA) procedure and the double range Duncan (DNMRT) test. The difference between mean values was considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

MYCELIUM, PINHEAD AND FRUITING BODY GROWTH ON CONTROL SUBSTRATES

Figure 1 shows the formation of mycelium (white fungus), pinhead, and fruiting body that filled the polypropylene bag (PP) space for the control mushroom substrate after 70 days of incubation. This mycelium formation is an indicator that the mushroom substrate had matured and was ready for the production of pinhead and fruiting bodies (Saidu et al. 2013). Based on the findings of the control mushroom substrate, the mycelium with

extracting pinhead had taken one day for the fruiting body to fully grow. The findings showed that the highest number of mushrooms in the substrate bag was four mushroom stems (Figure 1(d)), the least number of mushroom production was two (Figure 1(e)) and one big mushroom trunk (Figure 1(f)).

Commonly, mushrooms are understood as fruiting bodies of fungi that are edible (mushrooms) or poisonous (toadstools). They present a highly valuable food and serve as medicine to keep good health. Fruiting bodies as well as fungal mycelia may contain several bioactive compounds, and this biological entity is the key to a good efficiency and its productivity. However, the results of a different BE in this control samples were perhaps mainly due to lower substrate quality conditions (e.g. freshness consistency of all three main components of the substrates) and equally drastic physical property changes such as a decrease in the water content after the harvest which affected humidity, composition of atmospheric air and also air pressure (Kumari et al. 2015). Therefore, it is important to ensure that the moisture content of the mushroom substrate is always at its optimal condition (e.g. high RH: 70 to 80%; low temperature (<25 °C) and low CO₂ level) and satisfactory so that the next harvest of its regeneration phase can be carried out consistently.

MYCELIUM, PINHEAD AND FRUITING BODY GROWTH ON DIFFERENT TAPIOCA FLOUR CONCENTRATION MUSHROOM PRE-BLOCKS

Figure 2 and Table 1 show the findings of progressive vegetative growth and its timeline of mycelium, pinhead and fruiting body on the mushroom pre-blocks from 5% (w/v), 10% (w/v) and 25% (w/v) tapioca flour. Block substrate 5% (w/v) tapioca flour had produced three visible mushroom trunks (Figure 2(a)). This production showed that the use of tapioca flour with a concentration of 5% (w/v) was capable of producing mushrooms. However, block substrate with a concentration of 10% (w/v) tapioca flour did not produce a fruiting body but only mycelium on the block (Figure 2(b)). This was likely to be associated with the concentration of tapioca flour that affected the humidity of the pre-block as well as the mycelium movement and growth inside it as it became too cohesive throughout those fibrous substrates. Block substrate with 25% (w/v) tapioca flour produced a big mushroom trunk (set 1) whereas several big lumps of pin with anomalies looking growth were in set 2 (Figure 2(c)). The findings also showed that mushrooms were successfully grown by the production of a pinhead that yielded one visible consolidated big mushroom trunk.

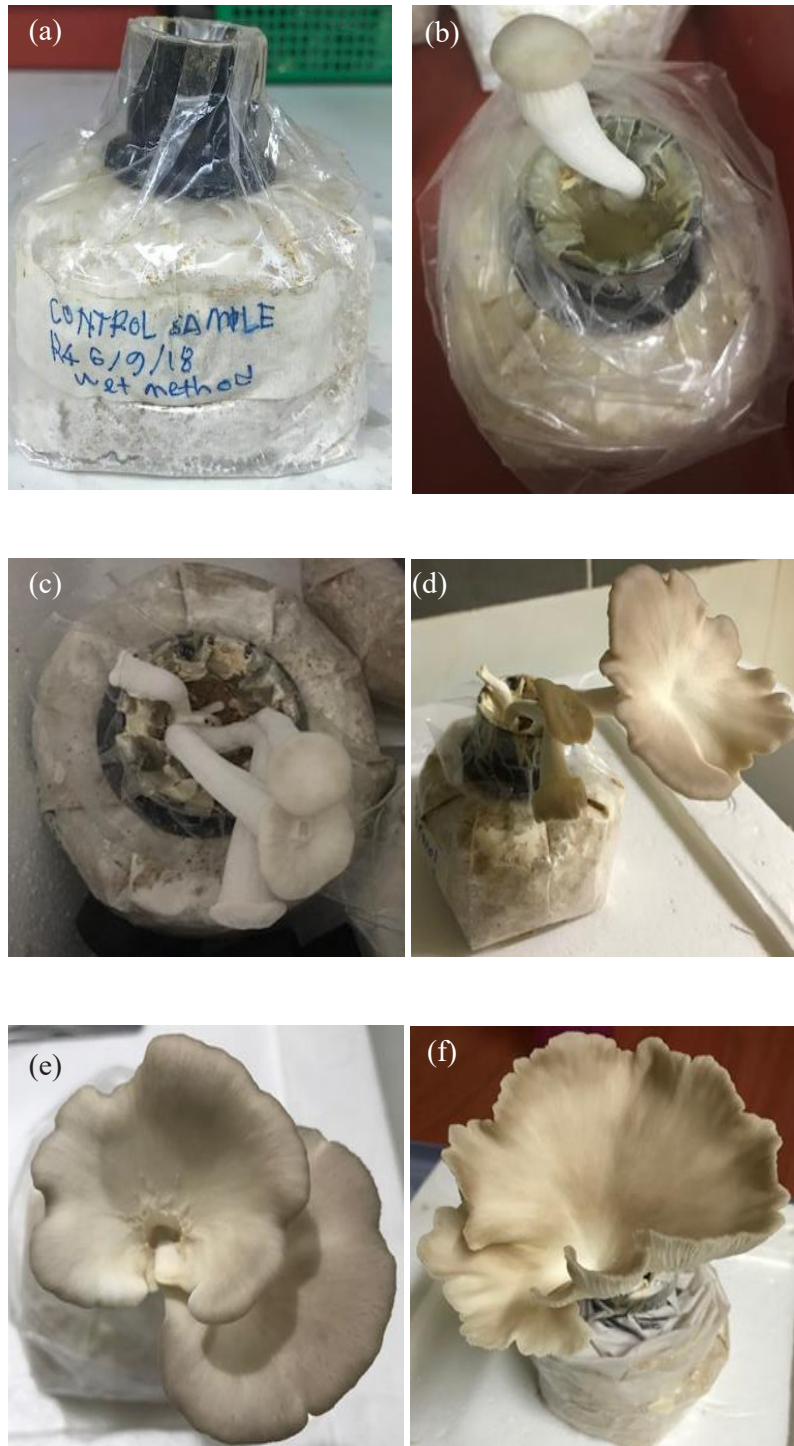


FIGURE 1. The growth profile (more than 2 months under aerial cooling of 25 ± 2 °C and relative humidity (RH) of 80-90%) of mycelium, pinhead and fruiting body from PP control mushroom substrate (: (a) substrate condition after 70 days of mycelium growth, (b) one pinhead (2 days post mycelium growth), (c) four pinheads (3 days post mycelium growth), (d) four fruiting mushrooms trunk (2 days post mycelium growth), (e) two fruiting mushrooms trunk (2 days post mycelium growth) and (f) one big fruiting mushroom (2 days post mycelium growth)

Nevertheless, a block of 25% (w/v) tapioca flour had also resulted in overly dense mould production. This is likely to be related to the density of tapioca flour used in the production of pre-blocks that affected its growth pattern.

Moreover, a growth timeline of mycelium in the control substrate for *P. ostreatus* production was merely 70 days. Whereas, the growth duration was 74 days for substrate pre-blocks with 5% (w/v) tapioca flour, while the block with 25% (w/v) tapioca flour took 80 days (Table 1).

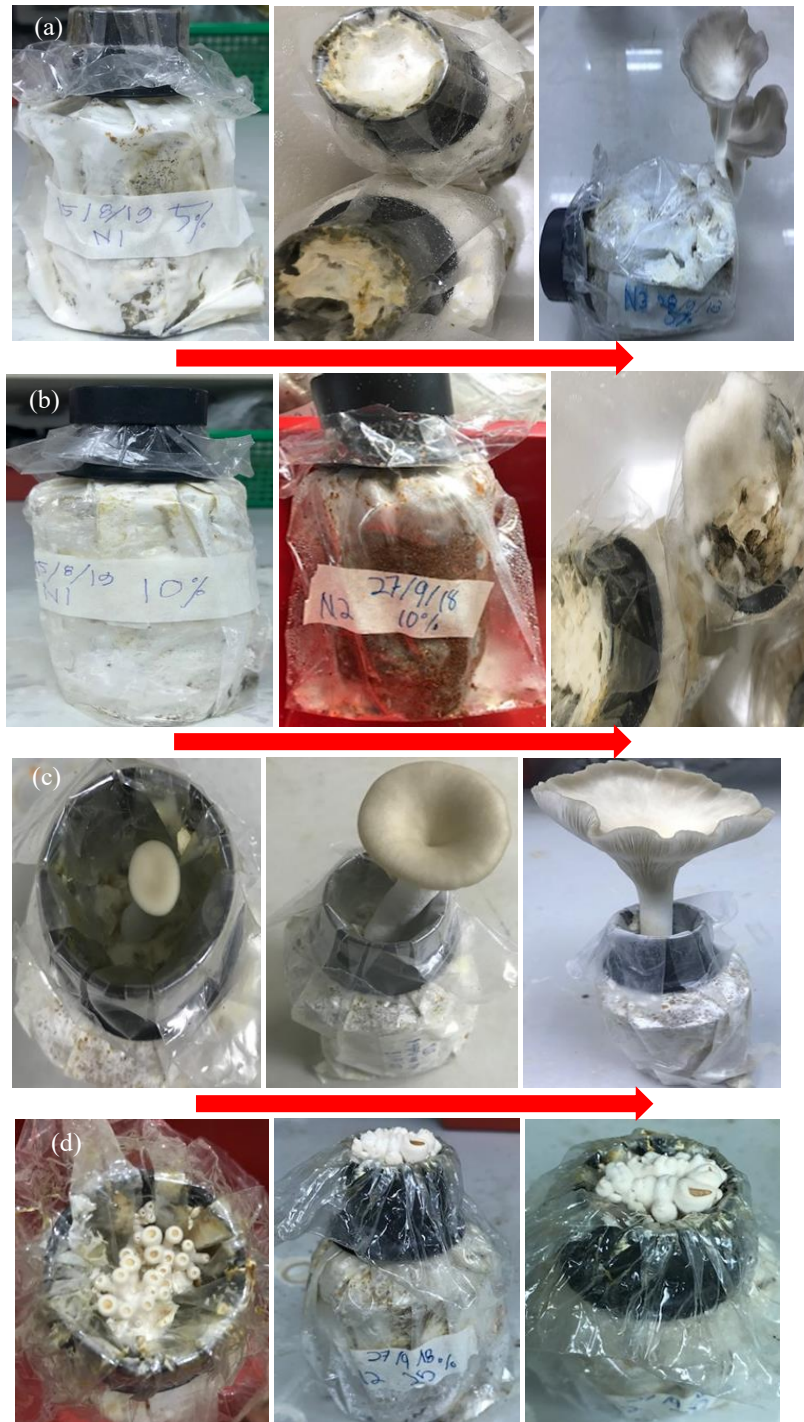


FIGURE 2. Progression fruiting body growth (red arrows: from mycelium growth (left) to fruiting (right)) of *P. ostreatus* from pre-block mushroom substrates of (a) S5%M: 5% (w/v) tapioca flour, (b) S10%M: 10% (w/v) tapioca flour, (c) S25%M: 25% (w/v) tapioca flour (set 1) and (d) S25%M: 25% (w/v) tapioca flour (set 2)

TABLE 1. Vegetative growth timeline of *P. ostreatus* on 3 different TF binder concentrations

Set	Substrate composition	The growth of mycelium	The growth of pinhead	The growth of fruiting body
(a) S5%M	100 g saw dust + 10 g bran + 1 g lime + 5% tapioca flour	74 days	3 days	2 days
(b) S10%M	100 g saw dust + 10 g bran + 1 g lime + 10% tapioca flour	73 days	5 days	2 days
(c) S25%M	100 g saw dust + 10 g bran + 1 g lime + 25% tapioca flour	80 days	3 days	2 days

MUSHROOM BIOLOGICAL EFFICIENCY

Table 2 shows total weight and its largest diameter growth in conventional polypropylene bags (PP) and various binder concentrations of mushroom blocks. The control produced lower BE (13%, w/w) than the blocks with binder ranging from 18 to 29% (w/w) ($p < 0.05$). The block substrate with 10% (w/v) tapioca flour did not produce potential BE (small pin heads and fruiting body were observed with inadequate vegetative growth) due to size of the prepared block was considerably quite small (which could hinder the vegetative growth) and the high density of the substrate used during pre-block production and compaction (e.g. human errors) which interfered with the mycelium movement which indirectly caused the ideal temperature not to be achieved during cultivation.

The variation observed was possibly due to the lower temperature setting ranging from 25 to 27 °C which ultimately affected its optimal growth. In fact, high temperatures in the cultivation macro-environment can also reduce the mushroom growth in different ideal

growth tracks. This allows for other microorganisms to adapt to higher temperature which leads to competing microbiota micro-environment and eventually affecting the essential fungi growth (Dias 2010). Nonetheless, lower temperature as well as dry condition reduced the stalk height and the mushroom diameter (Mahmud & Ohmasa 2008; Sher et al. 2010). Thus, the right temperature setting could be the cause of those variations as this experiment was carried out in the lab rather than in a well-controlled and conventional setting in the industrial scale farm. Moreover, the use of tapioca flour as a binder affected the weight of the mushrooms, mycelium movement and its imbalance growth consistently as compared to the conventional ways. The starch concentration used had slightly contributed to the substrate density which would affect the quality of the substrate. The temperature variation in the cultivation environment can also reduce the growth of mushrooms in different ideal growth tracks where this allows competition from other microbiota which are capable of adapting to higher temperatures (Dias 2010).

TABLE 2. Productivity of mushroom biological efficiency (BE) and the largest diameter observed

Substrate	Number of mushroom/bag	Largest diameter of mushroom (cm)	Total weight of mushroom (g)
+ve control (PP bag)	4–5	8.2 ± 1.1 ^b	13.21 ± 2.5 ^b
S5%M	3–4	10.3 ± 3.2 ^a	29.67 ± 5.6 ^a
S10%M	Small pin heads	NA	NA
S25%M	8–10	5.3 ± 1.2 ^c	18.40 ± 5.6 ^c

*-Mean with different alphabet shows significant difference between samples ($p < 0.05$); BE % was determined using the ratio of the total weight of the fresh mushrooms to the absolute dry weight of the substrates. NA: Not applicable. Only small pin heads with no further vegetative growth

BULK DENSITY AND WATER ABSORPTION OF
MUSHROOM PRE-BLOCK SUBSTRATE

Figure 3 shows the results of pre-block density test for three different types of tapioca flour concentrations (5% (w/v), 10% (w/v) and 25% (w/v)) as the binders mixed with the base material of the mushroom substrate (sawdust, rice bran and agricultural lime). The findings showed that the mushroom pre-block with a concentration of 25% (w/v) tapioca flour had a higher density than the ones with 10% (w/v) and 5% (w/v) due to starch density that bound the particles of substrate composition to make the blocks heavier and denser ($p < 0.05$). Figure 4 shows the water absorption (%) of the mushroom blocks for the three types of tapioca flour concentrations. The mushroom pre-block with the concentration of 5% (w/v) tapioca flour had higher water absorption ($p < 0.05$) due to lower consumption and concentration as compared to the 10% (w/v) and 25% (w/v).

The physico-chemical analysis of the pre-blocks substrates would perhaps be the preliminary model of a good recyclability and rigidity as compared to a conventional PP bags method. The first essential physical properties are pre-block bulk density post-compaction. The finding showed that 5 to 10% (w/v) TF mushroom pre-block had a wider and less dense pore that permitted more water to be absorbed as water holding capacity is heavily related to density (Choudhary et al. 2009). The compaction method affected density and this was a reflection of the compaction of the sample that could actually hinder the vital movement of the mycelium to vegetative fruiting growth (Annan & White 1998). Thus, the increasing substrate compaction had reduced the holding capacity of water, consequently affecting the heat and mass transfer coefficient in the substrate (Che Johari et al. 2017).

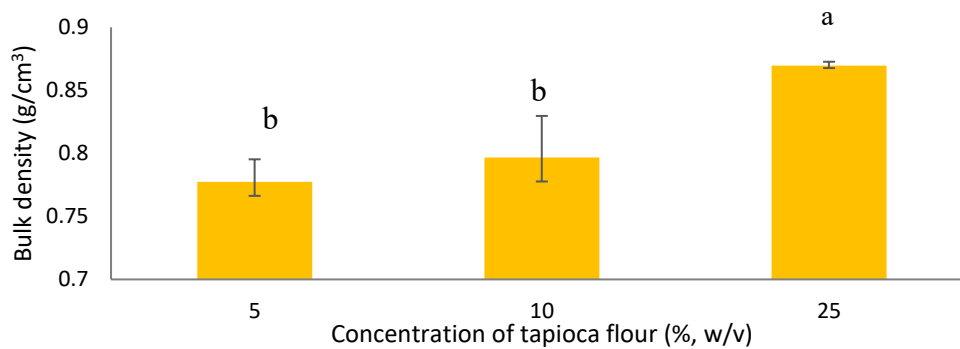


FIGURE 3. Bulk density of the mushroom block in different concentrations (% w/v) of tapioca flour. a-b: Mean \pm standard deviation between concentrations of tapioca flour that indicates a significant difference at $p < 0.05$

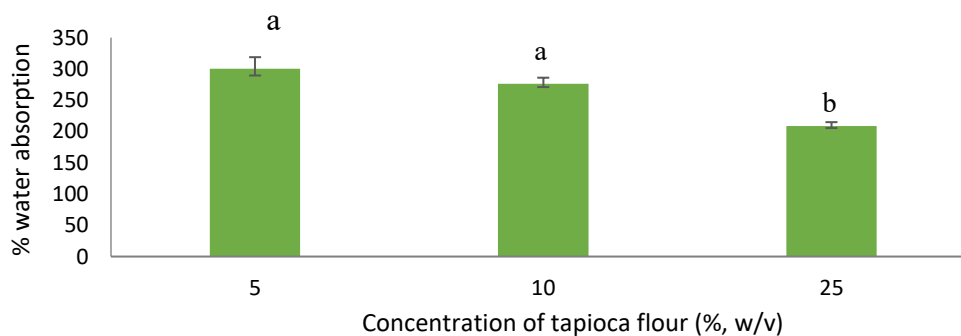


FIGURE 4. Water absorption (%) for mushroom block in different concentrations (% w/v) of tapioca flour. a-b: Mean \pm standard deviation between concentrations of tapioca flour that indicates a significant difference at $p < 0.05$

MUSHROOM PRE-BLOCK SUBSTRATE PH PROFILES

Figure 5 presents the pH readings for the pre-block substrate of the mushrooms before and after *P. ostreatus* seeding (spawning) where all substrates are either with mycelium or not producing the same profiles irrespective of different binder concentration used. For the mushroom substrate which was not filled with mushroom seeds, the pH indicated the reading rate between 8 and 9. Whereas, the mushroom substrate filled with the seeds recorded pH readings between 4 and 6. The reading was consistent throughout the increasing concentration of the binder as it profoundly affected the alkalinity of the substrates.

The effect of pH was one of the determinants of the productivity of the substrate for mushrooms (Mukherjee & Nandi 2004). The lowering of pH values in the substrate after seeding could be associated with the

use of different tapioca flour concentrations according to the predetermined block formulation as well as the substrate composition interaction with the tapioca flour and the seeds. However, as for the control substrate, the substrate composition interaction only occurred with water and it maintained at pH 5.5. The optimum pH substrate should be between 6 and 8 depending on the mushroom species (MushWorld 2004), and the optimum pH value for mycelium vegetative growth is between 5 and 6.5 (Mukherjee & Nandi 2004). Although mycelium endures at pH 4.2-7.5, the growth will decrease when the pH decreases and stops growing at pH 4. On the contrary, if the pH is higher than the optimum pH, mycelium growth will be faster but it will produce abnormal structure likewise. For that reason, the optimum pH value for the initial start and the formation of the fruit should be best ranging from 5 to 5.5 though it can occur at pH 5.5-7.8.

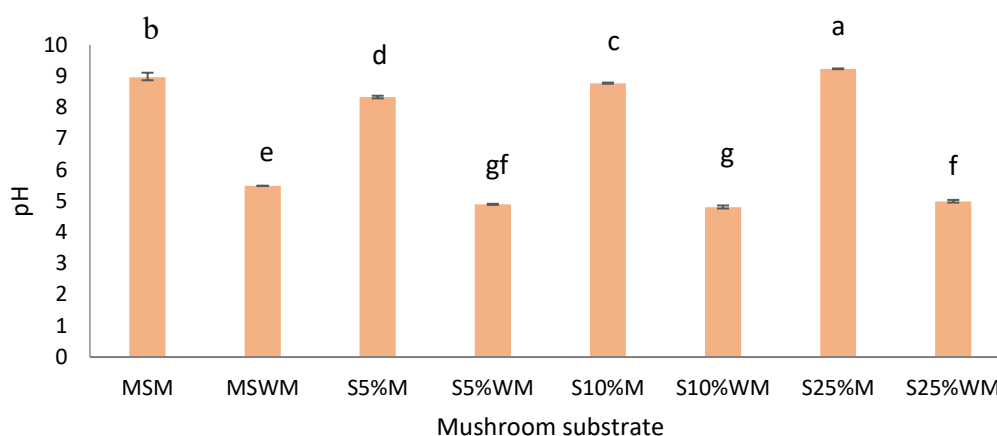


FIGURE 5. pH of the mushroom pre-block substrate before and after *P. ostreatus* seedings. MSM (+ve control): mushroom substrate with mycelium, MSWM (-ve control): mushroom substrate without mycelium, S5%M: substrate 5% TF with mycelium, S5%WM: substrate 5% TF without mycelium, S10%M: substrate 10% TF with mycelium, S10%WM: substrate 10% TF without mycelium, S25%M: substrate 25% TF with mycelium and S25%WM: substrate 25% TF without mycelium. a-g: Mean \pm standard deviation between pH values of the mushroom substrate that indicates a significant difference at $p < 0.05$

MUSHROOM PRE-BLOCK SUBSTRATE COLOUR PROFILES

Table 3 shows the colour comparison of the mushroom substrate with and without mycelium thread-like hyphae. The colour for eight types of mushroom substrate samples was measured with colour coordinate system values of L^* and a^* . The parameter L^* represents the brightness level of the analysed pre-block substrate. There was a significant difference between the colour of the mushroom substrates filled with mycelium (-ve control:

the brightest) and the ones with mycelium growth in 10% tapioca substrate (S10%WM; the lowest brightness) ($p < 0.05$). Meanwhile, a^* representing the red-green compositional colour profiles exhibited the highest value of 5.30 for the S25%WM as compared to the control (MSM and MSWM) ($p < 0.05$).

As for the substrate internal pre-block coloration, this would actually give an initial hypothesis that any additional foreign matters in the substrates (e.g. TF binder) would

trigger mushroom morphogenesis and increase levels of spawn ratio. Hence, high in brightness and red-green compositional colours (Table 3) may have resulted in yield (BE) increased as compared to the conventional PP bags (Table 2). The low water composition used in the control

substrate and the different TF binder concentration might have affected the redness and the yellowness/brightness (Saidu et al. 2013) that affected the coloration of the substrates collectively and the propagation of the mycelium towards the final fruiting stages.

TABLE 3. Colour profiles of mushroom pre-block substrates with mycelium and without mycelium

Sample	L*	a*
MSM (+ve control)	38.28 ^d	2.30 ^d
MSWM (-ve control)	51.26 ^a	3.92 ^c
S5%M	33.37 ^c	2.27 ^d
S5%WM	44.62 ^c	4.82 ^{ab}
S10%M	33.35 ^c	2.23 ^d
S10%WM	44.71 ^c	4.63 ^b
S25%M	34.07 ^c	2.24 ^d
S25%WM	47.81 ^b	5.30 ^a

MSM (+ve control): mushroom substrate with mycelium; MSWM (-ve control): mushroom substrate without mycelium; S5%M: substrate 5% TF with mycelium, S5%WM: substrate 5% TF without mycelium, S10%M: substrate 10% TF with mycelium, S10%WM: substrate 10% TF without mycelium, S25%M: substrate 25% TF with mycelium and S25%WM: substrate 25% TF without mycelium. a-e: Different letters indicate a significant difference at $p < 0.05$

COMPRESSIVE STRENGTH PROFILES

Figure 6 shows the compressive strength for the three types of mushroom block substrate using different binder concentrations of tapioca flour. The results showed that the concentration of the block binder with 25% (w/v) tapioca flour had higher compressive strength than the ones with 5% (w/v) and 10% (w/v) respectively ($p < 0.05$). The results were coherent with the increases of binder concentration as it provided extra rigidity to the porous structure due to its natural adhesiveness of interlocking any fibrous material. Moreover, it had also been found that compressive strength of the pre-block was influenced by the ratio of tapioca flour content in the mushroom substrate and its hydration level. This is possibly due to the fact that when the content of tapioca flour concentration decreased, the absorption increased while the strength decreased in which the blocks with high water absorption had lower strength (Aizad et al. 2021; Basar & Aksoy 2012).

MUSHROOM PRE-BLOCK SUBSTRATE CRUDE PROTEIN CONTENT

Based on the analysis, it was found that the crude protein content of the substrates was in the range of 1-1.7% (Figure 7). The highest crude protein content was found in the control mushroom substrate without mycelium (MSWM) at 1.73% ($p < 0.05$) indicating the remnants of total protein availability from rice bran after 2 months of vegetative growth. In fact, the depletion of protein sources was noticeably consistent in the pre-block substrates with mycelium indicating the nitrogen-rich material has been utilised to some extent for the vegetative growth. As for its nutritional quality (pre-block substrates), the differences between S5%M and S25%M as compared to the control (MSWM) indicate the fungus was consuming well on the readily available protein from rice bran as its main nitrogen-rich sources ($p < 0.05$). Therefore, the crude protein content of the *Pleurotus* depended profoundly on the substrate nutritional composition and

the mushroom species as the produced mushroom are generally reliable alternative high crude protein sources which is usually 20-30% of dry matter (Deepalakshmi & Mirunalini 2014; Erjavec et al. 2012).

MUSHROOM PRE-BLOCK SUBSTRATE CRUDE FAT CONTENT

The crude fat content (%) of all mushroom substrates ranged from 0.1 to 1.80% with the highest level of crude fat content found in 10% TF mushroom substrate with mycelium (S10%M) (Figure 8). The other set of samples were considered within the normal range (0.1-0.3 g) of fat content in any mushroom. The high fat content in S10%M might be due to excessive amount of TF used during the pre-block production that could exceedingly affect the normal mushroom vegetative growth. A consistent low crude fat content (2-3%) was reported as mushrooms are generally low in oils and fats although they may contain some essential fatty acids and can be suggested as good supplements for patients with heart problems (Deepalakshmi & Mirunalini 2014). Moreover, the high in crude protein and low-fat contents of the various *Pleurotus* species were enhanced by the different agro-wastes used for planting (e.g. industrial and agricultural lignocellulose-wastes of coffee pulp, coffee waste, cotton stalks and paper) and this would absolutely contribute to the nutritional content of the mushroom that possess both nutritional and medicinal attributes (e.g. dietary supplement for therapeutic purposes) (Ali et al. 2009).

MUSHROOM PRE-BLOCK SUBSTRATE CRUDE FIBRE CONTENT

The overall crude fibre contents (%) of eight mushroom substrates ranged from 9.6 to 13% and consistently the same in all different TF binder concentrations (Figure 9). The highest content of crude fibre was found in both mycelium control substrates (MSM and MSWM) ranging from 12 to 13.32% ($p < 0.05$). The analysed crude fibre content was considered as totality of the crude fibre containing in the mycelium and the substrates itself as the normal mushroom crude fibre content of that only 7-8%. The high amount of crude fibre content of -ve control substrate as compared to the others was mainly attributed by the non-existing mycelium in the substrate containing sole material of sawdust as its main fibre sources. The addition of starch binders as part of the substrate compositions in the cultivation process has slightly reduced the crude fibre content in all TF binder substrates ($p < 0.05$). Therefore, the results of the nutritional composition of the substrate and fruiting body were in line with the prior study (Silva et al. 2012) that there was a chemical composition (e.g. polysaccharide and phytochemicals) correlation in the mushrooms and also in the substrate used for cultivation. However, the chemical composition of the mushrooms will indeed be affected with respect to different chemical substrate composition, and yet its nutritional composition of the fruiting body will absolutely differ when grown on different substrate configuration (Khan et al. 2008; Sheu et al. 2007).

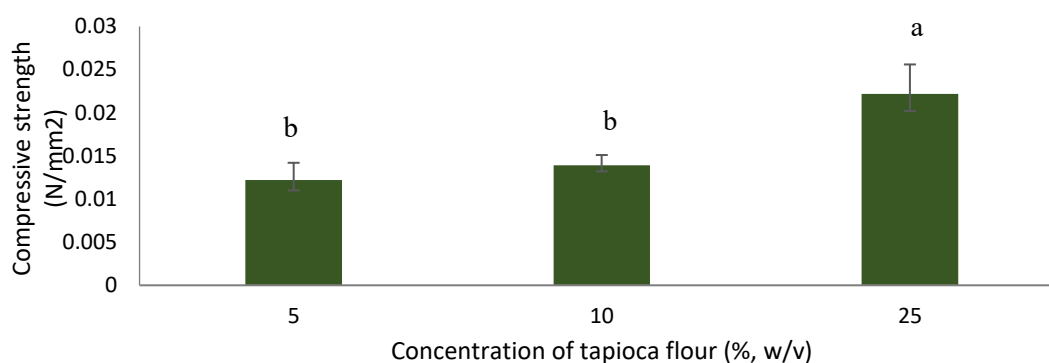


FIGURE 6. Pre-block substrate compressive strength test versus different concentration (% w/v) of tapioca flour. a-b: Mean \pm standard deviation between concentrations of tapioca flour that indicates a significant difference at $p < 0.05$

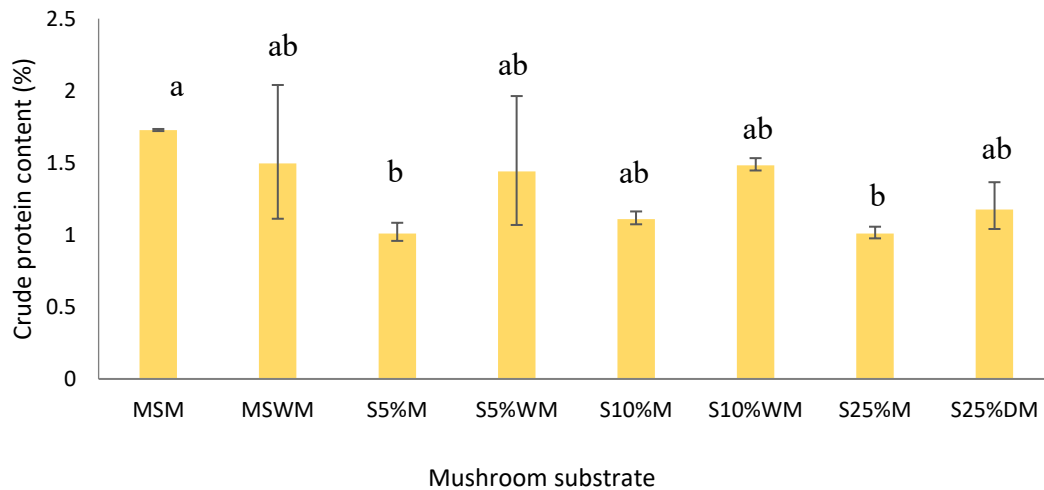


FIGURE 7. Crude protein content (%) in mushroom pre-block substrates. MSM (+ve control): mushroom substrate with mycelium, MSWM (-ve control): mushroom substrate without mycelium, S5%M: substrate 5% TF with mycelium, S5%WM: substrate 5% TF without mycelium, S10%M: substrate 10% TF with mycelium, S10%WM: substrate 10% TF without mycelium, S25%M: substrate 25% TF with mycelium and S25%WM: substrate 25% TF without mycelium. a-b: Mean \pm standard deviation between protein contents of mushroom substrate that indicates a significant difference at $p < 0.05$

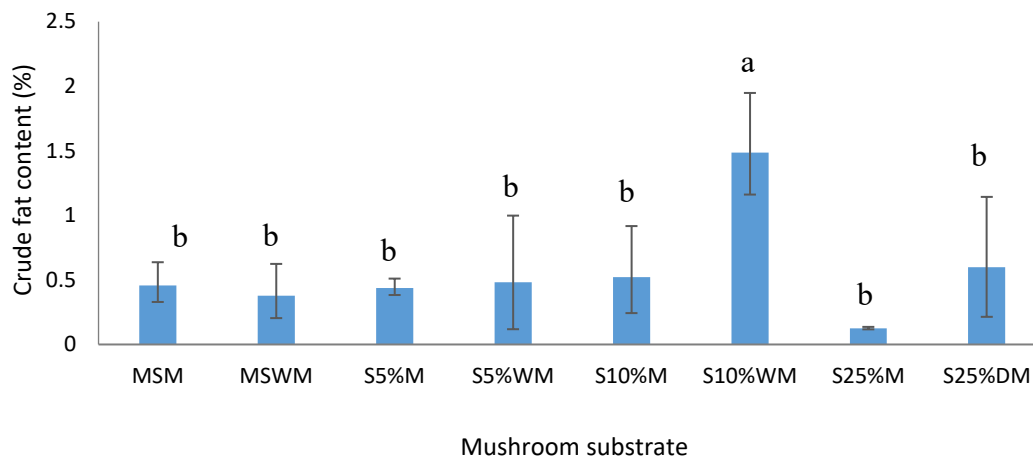


FIGURE 8. Crude fat content (%) in mushroom pre-block substrates. MSM (+ve control): mushroom substrate with mycelium, MSWM (-ve control): mushroom substrate without mycelium; S5%M: substrate 5% TF with mycelium, S5%WM: substrate 5% TF without mycelium; S10%M, substrate 10% TF with mycelium, S10%WM: substrate 10% TF without mycelium, S25%M: substrate 25% TF with mycelium and S25%WM: substrate 25% TF without mycelium. a-b: Mean \pm standard deviation between fat contents of mushroom substrate that indicates a significant difference at $p < 0.05$

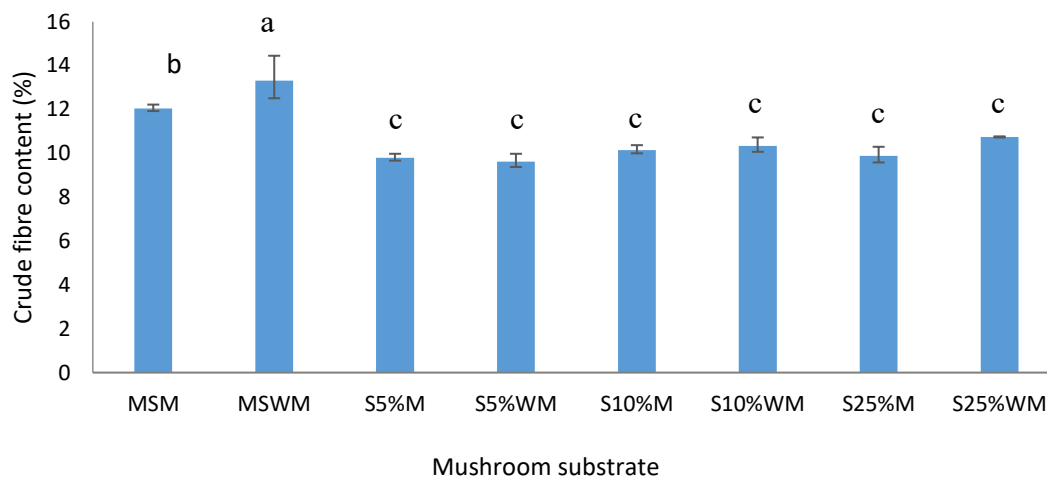


FIGURE 9. Crude fibre contents (%) in mushroom pre-block substrates. MSM (+ve control): mushroom substrate with mycelium, MSWM (–ve control): mushroom substrate without mycelium, S5%M: substrate 5% TF with mycelium, S5%WM: substrate 5% TF without mycelium, S10%M: substrate 10% TF with mycelium, S10%WM: substrate 10% TF without mycelium, S25%M: substrate 25% TF with mycelium and S25%WM: substrate 25% TF without mycelium. a-c: Mean \pm standard deviation between fibre contents of mushroom substrates that indicates a significant difference at $p < 0.05$

CONCLUSION

The use of tapioca flour as a binder material affected the nutritional composition of the substrate as compared to the conventional method besides having a sturdy pre-block substrate. Even though the physico-chemical analyses of density, water absorption, compressive strength test, pH and colour values exhibited a good and ideal micro-environment growth of the mushroom, some nutritional deficiencies were observed though imperatively acceptable as compared to the conventional method of compressed substrate in polypropylene bags (PP). Hence, the mushroom cultivation method through the formation of pre-block substrate bound together with tapioca flour exhibited a worthy mushroom production (e.g. biological efficiency and robustness) which was potentially established in the range of 5-25% (w/v) tapioca flour concentrations. Further studies are needed to decide on the best concentration for optimal mushroom growth without compromising the pre-block substrate structural integrity and more importantly its recyclability and biological efficiency being attained.

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