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Growth performance and mineral analysis of *Pleurotus ostreatus* (oyster mushroom) cultivated on spent mushroom medium mixed with rubber tree sawdust

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ABSTRACT

The objective of this paper is to study potential application of spent mushroom substrate (SMS) in production of *Pleurotus ostreatus* mushroom. A various mass ratio mixture of rubber sawdust (RS) and SMS with wheat bran supplement were prepared for mushroom cultivation. The mushroom house was kept at an optimal condition with temperature between 28 and 33 ℃ and humidity in the range between 80 and 95%. The results showed that the 50% SMS + 50% RS had a significantly higher of average total yield at 156.12 g/bag, biological efficiency (B.E) of 55.76% and required only 26 ± 2 days for mycelium growth in comparison to 100% RS, a control substrate. It is also found that potassium (K) was the most concentrated macroelement and all heavy metal concentrations were below the maximum permitted by WHO/FAO in the first fresh mushroom collected. Therefore, it can be proven that SMS can be a great solution to the disposal issue, and it can be an excellent medium for the cultivation of *Pleurotus* spp. when it is combined with the RS.

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1. Introduction

Pleurotus species are popular and are cultivated worldwide, particularly in Asia, America and Europe [1] and are good for health because of their richness in protein, fiber, minerals and also vitamins [2]. *Pleurotus* are saprophytes and they extract the nutrients from their medium through its mycelium to obtain elements essential to its growth, such as nitrogen (N), carbon (C), minerals and vitamins [3]. Phosphorus (P), magnesium (Mg) and potassium (K) are also taken into consideration as mushroom macronutrients, and trace elements such as selenium (Se), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and molybdenum (Mo) appear to be crucial for various functions [4].

Spent mushroom substrate (SMS) can be defined as the biomass produced by the mushroom industry that is left after a mushroom crop is harvested [32]. For one kilogram of mushroom produced, about five kilograms of SMS is generated [5]. In Malaysia, the production of mushrooms per month is about 8424 metric tonnes and consequently the accumulation of SMS amounts to 42,120 metric tonnes per month [5]. An enormous amount of SMS is added to the burden of municipal rejections, particularly around the mushroom cultivation farms.

SMS are usually regarded as agricultural waste and are typically disposed through spreading on land, open burning or disposed in situ, composting with animal manure, landfilling, or burying [6] without being utilized properly. This is a critical challenge as improper disposal could result in environmental problems like soil contamination, and air and water pollution [7]. In the future, more effective as well as efficient use of SMS is necessary to prevent the spread of environmental conservation, fungal diseases, waste recycling, an improved agricultural economy and a sustainable mushroom industry [8]. Reusing or recycling could serve as an effective method in mitigating this solid waste. To reduce the environmental impacts that arise from mushroom production, SMS could be reused or recycled for a new *P. ostreatus* cycle. The use of SMS to grow *P. ostreatus* also helps to alleviate the increasing difficulty of obtaining the declining supply of RS.

According to Ashrafi et al. [9], SMS does not produce good performance when employed for the second time in the production of

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mushroom as a growing medium, due to the repeated use of nutrients by mycelium which has resulted in nutrient depletion. Whereas, mushrooms are dependent on substrate for nutrition. The substrate's nature as well as nutrient composition impacts the mushroom quality and the crop performance of the biotransformation process [10]. Mycelium growth as well as fruiting body production of *Pleurotus* spp. are impacted by the cellulose, hemicellulose and lignin compositions and the ratios of carbon/nitrogen [3]. As a result, nutritional supplementation of the mushroom substrate is a crucial cultural practice of growing mushrooms. In fact, supplementation of the substrate with different ingredients is suggested before spawning to improve the yield of mushrooms. Rice bran, wheat bran and egg shell powder have been widely employed as food supplements in order to boost the yield, biological efficiency, and growth through the supply of adequate N and slow-release nutrients. Substrate supplementation with high-protein materials has been proven to improve the performance of *Agaricus*, *Pleurotus* and *Lentinulastrains* [9]. Thus, SMS nutritional supplementation can be a solution to enrich SMS to produce a respectable yield of mushroom.

In Malaysia, there is a lack of comprehensive works on the performance of various oyster mushroom species grown using the agricultural byproducts, grasses, and wastes, as substrates. In addition, there also is limited evidence on the mushroom waste usage in growing mushrooms again. Therefore, in order to fill the gap, this article presents the utilization as well as recycling of SMS as the mushroom substrate for the new crop cycle. This study provides an economic and ecological approach to the use of SMS for the production of *P. ostreatus*, by which the SMS biomass can be effectively transformed into high quality edible mushroom. This study's aim is to examine the effectiveness of the growth performance of *P. ostretaus* by reutilizing the commercial SMS from *Pleurotus* spp. as a base medium with supplement which is wheat bran for further *P. ostreatus* production by analyzing biological efficiency (B.E) as well as yield of the fruiting body and to determine the mineral content of the fresh mushroom grown using SMS mixed with RS.

2. Materials and methods

2.1. Location and experiment conditions

This research was conducted between January and December 2020 under a 0.6 m × 0.6 m canopy, in an exposed space near Block Y, at Universiti Malaysia Pahang which is situated at 3.5437° N, 103.4289° E. The local environment was warm, moist as well as tropical with much rains. The mushroom room was positioned under the canopy. The canopy was layered with 70% solar nets to prevent extreme direct sunlight getting in the mushroom room. This framework was resembled to that utilized via local growers. One rack was allocated for each mushroom house. The mushroom room was complete with four layers of steel shelves and there was 50 cm spacing between the steel shelves that served as walkway access for human. There were 10–12 growth beds for each shelf layer and a total of 40–48 growth beds per rack. The humidity and temperature of the mushroom were measured using a LCD Digital Hygrometer Temperature Humidity Meter. The humidity and temperature inside the mushroom house were kept in an optimal condition with temperature between 28 and 33 °C [11] and humidity in the range between 80 and 95% [11]. The *P. ostreatus* was cultivated using SMS mixed with RS. The spawn of *P. ostreatus* was obtained from the Pekan Mushroom Resources Sdn. Bhd., Pekan, Pahang. The Pekan Mushroom Resources Sdn. Bhd., Pekan, Pahang is situated at 3.4836° N, 103.3996° E. The spawn of *P. ostreatus* was made from sorghum material.

2.2. Preparing raw materials

RS, SMS, wheat bran as well as calcium carbonate have been collected from local farmers from Pekan Mushroom, Pekan, Pahang. The SMS has been dried and shredded into smaller sizes using a shredder at the Laboratory of the Faculty of Chemical and Process Engineering, Universiti Malaysia Pahang. The RS and SMS were sieved using a mesh tray (1 mm) to eliminate impurities and to have consistent size of substrate particle.

2.3. RS and SMS characterizations

The ultimate analysis was carried out in determining the elements present in the RS and SMS. The CHNS Elemental Analyzer (Elementar, Japan) at the Central Laboratory, Universiti Malaysia Pahang (UMP) was utilized for the analysis according to ASTM D5373-02 for determining the carbon to nitrogen ratio of the mushroom medium employed in this work. The oxygen supply was kept at a pressure between 2 and 2.2 bar while the helium intake was maintained at a pressure of 1.2 to 1.25 bar with a flow rate of 600 mL/min. Approximately 20–200 mg of sample was weighed in tin foil and placed on the automatic vario Macro cube Elementary sampler. The sample was combusted at 1, 150 °C and the resulting gases were analyzed.

The thermal degradation analysis of RS and SMS was performed using Thermogravimetric Analysis (TGA) (STA-7000, Hitachi, Japan) at the Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang in order to determine the moisture content, hemicellulose content, cellulose content, lignin content and ash content of the RS and SMS samples. Air and nitrogen gases are used to purge the equipment in simulating conventional combustion at 50 mL/min and 10 °C/min flow rate and heating rate, respectively. The experiment was performed at a temperature between 30 °C and 900 °C with nitrogen flow. The temperature was then adjusted to 900 °C for 30 min with air flow. The degradation of the lignocellulosic structure begin at 200 °C and ended at a temperature greater than 600 °C.

Metal composition of RS and SMS in terms of elements were also identified by using X-ray Fluorescence (XRF) (ZSX PRIMUS II, RIGAKU, United Sate) at the Centre Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang. The XRF has been initiated to increase the generator setting to 50 kV, 50 mA.

2.4. Preparation, inoculation and incubation of substrates

Five various compositions of substrate, with three replicates for each substrate composition were prepared, as indicated in Table 1. A 100% RS substrate was employed as control substrate. All substrates were blended with calcium carbonate (1.5 wt%) and wheat bran (5 wt%). In order to increase the substrate media moisture content, the clean water was then added into the blending substrate until the water was fully absorbed. The 20 × 30 cm polypropylene bags were filled with wet substrates with a 15 cm height in order to have constant volume of 1178 cm^3. The prepared substrates were formerly compressed and packed tightly, sealed with PVC-neck that were covered with paper to avoid the insects. The baglogs were sampled to measure moisture content, pH and C/N ratio. The baglogs loaded with substrates were sited on the upright steel racks and then sterilized for eight hours at 100 °C in the sterilization chamber. The baglogs were cooled to ambient temperature prior to inoculation after sterilization.

Table 1
Composition of the substrate on a dry basis.

Composition of mushroom substrate (%)
100 % SMS
25 % RS + 75 % SMS
50 % RS + 50 % SMS
75 % RS + 25 % SMS
100% RS

2.5. Cultivating and harvesting mushrooms

After sterilization, the substrate-filled bags were inoculated with 7–10 g of spawning seeds spread across the substrate surface where the baglogs were shaken thoroughly for appropriate spawning distribution the under clean environments. These bags were moved in vertical position into the dark room for spawn cycle where the temperature was held constant at 28 °C and the relative humidity was set to be in the range of 80–85%. The bags were moved to the harvest room after the mushroom substrate became white due to fungal mycelium colonization. The baglogs were reorganized horizontally and the top sections were opened for inducing fruiting body for the initial cropping. Water was sprayed to the baglogs to control the temperature and to retain the moisture condition. The development of pinhead till mushrooms reached normal size were observed and determined. Each baglog was harvested separately and the weight of fresh mushrooms per flush was recorded. After harvesting, the cropped bags were closed until the subsequent harvest. There were three harvests collection was done in this study.

2.6. Mycelium growth, average total yield and determination of biological efficiency (B.E)

The data were recorded according to the number of days desirable for the mycelium to colonize completely, for the initiation of primordia and for the first harvest. The weight of the fresh mushroom was determined. After three harvests, the data accumulated were employed to compute the biological efficiency (B.E) as well as average total yield. Total average yield in g/bag was calculated as the average total yield from three flushes with three replicates for each composition. Biological efficiency (B.E) was determined from the average total yield of *P. ostreatus* accumulated per dry weight of substrate employed in percent as displayed in (Eq. (1)).

$$\text{B.E}\ (\%) = \frac{\text{Fresh weight of mushroom per bag (g)}}{\text{Dry weight of substrate per bag (g)}} \text{ x } 100\ \% \quad (1)$$

2.7. Mineral testing of the fruiting body

The mineral element (Ca, Mg, K and Na) as well as the heavy metal element (Cu, Pb and Cd) in the bodies of fresh mushroom resulted from the initial crop that grown on 100% RS and 100% SMS were determined by using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (7500a, Agilent, Japan) at Central Laboratory, Universiti Malaysia Pahang. The mineral element (Ca, Mg, K and Na) as well as heavy metal element (Cu, Pb and Cd) were analyzed according to CENLAB/WI/CHEM-TM/008 method. Standard reference materials have been employed for quality control and assurance, and treated in a similar manner to samples during the test.

2.8. Statistical analysis

The experiment was completely randomized design (CRD) with three replications and five treatments. Regression analysis was performed to assess the trend across the substrates. The trends were either fitted to linear or quadratic. All statistical analysis was carried out by using the Microsoft Excel Software 2019.

3. Results and discussions

3.1. Characterization of RS and SMS

Mushroom needs a well-balanced nutrient within the substrate as a carbon to nitrogen ratio. The total amount of carbon (C) in the C/N ratio stands for the carbon content particularly in the form of hemicellulose, cellulose and lignin [12]. The carbon to nitrogen ratio (C/N) of spent mushroom substrates (SMS) and rubber tree sawdust (RS) are shown in Table 2. Table 2 tabulates that the RS had the greatest carbon to nitrogen ratio which was 181.82 compared to SMS which was 64.64. The value of C was slightly greater in the substrate formula 100 % RS (40 %) than SMS (39.43 %). It also can be seen that the substrate composition of 100 % SMS had 0.61 % total of N whereas 100 % RS (control substrate) had the total N of 0.22 %. Chang and Miles [13] recommended that a C/N ratio of 32 to 150 as the most suitable ratio for producing *P. ostreatus*. It is because the carbon source is a key component of storage and structural compounds in the cell and it is present in the form of monosacharides, disacharides, and polysaccharides. Nitrogen is a crucial element needed by all fungi to synthesize compounds containing nitrogen like pyrimidines, purines, protein and for chitin, which is the cell wall component composed of β(14)-linked unit of N-acetylglucosamin [14]. The C/N ratio had greater impacts on mycelium development, and also formation as well as growth of the fructifying body [14].

The hemicellulose, cellulose and lignin content in the SMS was studied using the thermogravimetric analysis (TGA) as shown in Fig. 1. The first stage of weight loss for both SMS and RS was at <150 ℃, which was due to the moisture content. It is very difficult to distinguish between hemicellulose and cellulose. However, according to Yahaya et al. [15], the degradation of cellulose and hemicellulose can be determined at temperature ranging from 150 to 380 ℃. Thus, a 63 % weight reduction of cellulose and hemicellulose was found in RS and a 53 % weight reduction in SMS at 150–380 ℃. The content of cellulose and hemicellulose in RS is slightly higher than that of in SMS. The weight reduction for lignin

Table 2
C/N ratio of RS and SMS.

Elements (wt %)	Raw materials	
	SMS	RS
Carbon	39.43	40.00
Nitrogen	0.61	0.22
Hydrogen	3.91	7.42
Sulphur	6.73	2.49
C/N Ratio	64.64	181.82

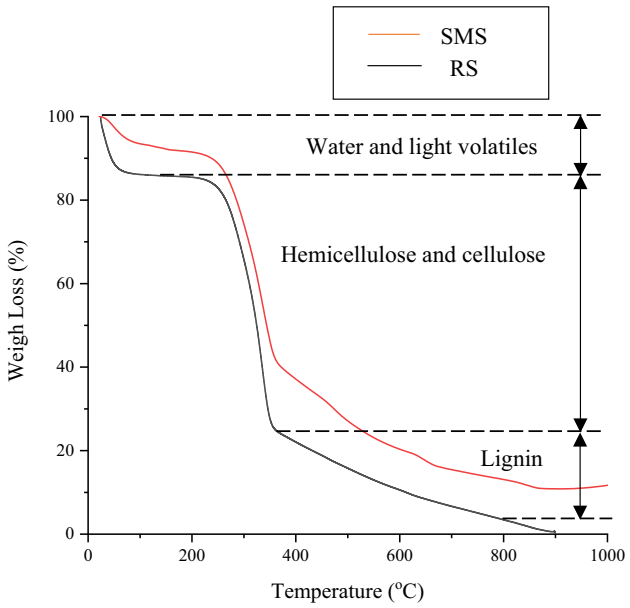

Fig. 1. Thermogravimetric (TGA) curve of RS and SMS.

is determined at temperature ranging from 380 to 800 ℃ where 18 % weight reduction of lignin was observed in RS and 26 % weight reduction was observed in SMS. The weight loss at >800 ℃ was due to ash content. There was no trace of ash in RS but 10 % weight reduction of ash was found in SMS. Hu et al. [16] claimed that cellulose serves as an energy source for fungal growth and metabolism. Lignocellulosic content which consists of hemicellulose, cellulose and lignin is very important as a source of nutrient in mushroom cultivation. It can be deduced that there is no significant lignocellulosic content in RS and SMS. Thus, SMS can be reused for further mushroom production.

X-ray fluorescence spectrometer (XRF) analysis of the rubber tree sawdust (RS) and spent mushroom substrate (SMS) samples provided a complete analysis of the elemental composition and all the chemical elements contained in the samples. Table 3 tabulates the macronutrients as well as trace elements obtained in the RS and SMS samples. The existence of both macronutrients and trace elements is extremely small amount, which is less than 3 % and they consist of magnesium (Mg), calcium (Ca), potassium (K), iron (Fe), phosphorus (P), sodium (Na), manganese (Mn) and zinc (Zn). The quantity of macronutrients existing in the RS and SMS complied with the order of P < Mg < K < Ca while trace elements complied with the order of Zn < Na < Mn < Fe. The existence of these minerals in the substrates is essential for the development of mushroom [17].

3.2. Physicochemical properties of substrates

The total C, total N contents, moisture content and pH as well as carbon to nitrogen ratios of different mushroom substrate are really essential aspects for mycelium colonization as well as the fruiting bodies' growth, which are presented in Table 4. The greatest moisture content was discovered in 50 % SMS + 50 % RS followed by control substrates (100 % RS), 100 % SMS, 75 % SMS + 25 % RS and 25 % SMS + 75 % RS, in that order. In this existing work, the moisture content in the 50 % SMS + 50 % RS substrate was greater as contrasted to other formulations. This could be because of the 50 % SMS + 50 % RS substrate having better water holding capability than other growing substrates. According to Bellettini et al. [3], moisture content is an extremely critical aspect that influences the growing of *P. ostreatus* mushrooms, as it influences the production as well as yield of the mushroom. In earlier researches, substrate moisture content has been readjusted between 50 and 75 % [18]. In present work, the moisture content of the substrates was approximately in the range between 58.35 and 63.55 %. Same procedure was also done by Ryu et al. [12] who cultivated *P. eryngii* at 65–68 % of moisture. Moisture content over 70 % allows the grow of contending diseases and moulds [3]. The pH values for mushroom substrate utilized in this present work varied from 6.27 to 7.67 which resembles the pH range made use of by Hoa et al. [14]. It is reported that in solid-state fermentation, the pH of the substrate was adjusted to 6.0 for *P djamor* [19]. It can be seen that highest C content was found in the 75 % SMS + 25 % RS. Meanwhile, the total N content of the substrates enhanced progressively as the amount of SMS in the substrate formula increased. In the present work, the carbon to nitrogen ratio of the substrate formula varied from 104.4 to 126.5 and the highest C/N ratio was observed in the 100 % RS substrate. The carbon to nitrogen relationship depended on both carbon and nitrogen sources. In current research, high C/N ratio of the control substrate (100 % RS) enhanced yield where an excellent C/N ratio of 126.5 was obtained from the control substrate which is 100 % RS.

3.3. Durations to complete mycelia growth, pinhead formation and first fruiting body harvest

All growth variables identified in this study were not significantly impacted by the different formulation of substrates as shown in Table 5 and Table 6. All the replicates for all substrates showed no difficulty for the mycelium to fully colonize the bags. The addition of RS or the mixture of RS and wheat bran did not have a significant impact on the duration required to complete mycelium development, duration required for the first pinhead formation and duration required for first fruiting body harvest as shown in Table 5. In Table 5, the colonization of oyster mushroom was completed between 24 ± 3–30 ± 4 days after incubation for all substrates. The control substrate, which is 100% RS, had the shortest time of 24 ± 3 days to complete the spawn running because of easy digestion and rapid decomposition of RS. The second shortest time is observed on the substrates 25 % SMS + 75 % RS (25 ± 2 days), followed by 50 % SMS + 50 % RS and 75 % SMS + 25 % SMS which took around 26 ± 2 and 28 ± 2 days, respectively. The 100 % SMS took the longest time (30 ± 4 days) for the bag to be fully colonized by mycelium. The number of days necessary for the colonization of a particular substrate varies depending on the fungal strain, growing conditions and substrate type [20]. This variation may be attributed to chemical composition variations, lignocellulosic

Table 3
Percentage of macronutrients and micronutrients present in the rubber tree sawdust (RS) and spent mushroom substrate (SMS).

Raw material	Macronutrients				Micronutrients			
	Ca	Mg	P	K	Na	Fe	Zn	Mn
	(Mass %)							
Rubber tree sawdust (RS)	0.34	0.09	0.04	0.28	0.00	0.01	0.002	0.007
Spent mushroom substrate (SMS)	2.28	0.41	0.23	0.90	0.01	0.02	0.002	0.015

Table 4
pH, moisture content (%), Total C (wt %), Total N (wt %) and C/N ratio on different composition of SMS in substrate (%).

Composition of mushroom substrate (%)	moisture content (%)	pH	Total C (wt %)	Total N (wt %)	C/N ratio
100% RS	62.54	6.27	17.71	0.14	126.5
25 % SMS + 75 % RS	56.95	7.13	24.97	0.22	113.5
50 % SMS + 50 % RS	63.55	7.36	25.49	0.23	110.8
75 % SMS + 25 % RS	58.35	7.67	27.1	0.24	112.9
100 % SMS	60.22	7.54	26.11	0.25	104.4

Table 5
Average days taken to complete mycelia growth, days taken for pinhead formation and days taken for first fruiting body harvest on different composition of SMS in substrate (%).

Composition of mushroom substrate (%)	Days taken to complete mycelia growth (days)	Days taken for first pinhead formation (days)	Days taken for first fruiting body harvest (days)
100 % RS	24 ± 3	27 ± 3	29 ± 2
25 % SMS + 75 % RS	25 ± 2	28 ± 2	30 ± 2
50 % SMS + 50 % RS	26 ± 2	29 ± 2	31 ± 2
75 % SMS + 25 % RS	28 ± 2	31 ± 2	33 ± 2
100 % SMS	30 ± 4	33 ± 4	35 ± 2

component and carbon to nitrogen ratio of the substrate. In this work, the control substrate, which is 100 % RS, had the relatively higher C/N ratio (181.82) than the spent mushroom substrate (64.64). This may significantly affect the number of days needed to completely colonize the mycelium in the substrate. Meanwhile, the pinhead formation after the substrate is fully colonized was around 3 days for all substrate formulations, as shown in Table 5. Pinhead formation basically is the mycelia growth's second stage in mushroom cultivation [21]. Although Patel et al. [22] found that oyster mushrooms required 16–20.33 days to fully colonize the substrate and 20.33–25.33 days to initiate the pinhead in the wheat straw substrate. It is found that there was no significant difference in the duration of pinhead formation up to the formation of the fructification body where mature mushrooms usually can be seen within 2–4 days after pinhead formation [33].

Table 6
Average total yield (g/bag) and biological efficiency (B.E) on different composition of SMS in substrate (%).

Composition of mushroom substrate (%)	Average total yield (g/bag)	Biological efficiency (B.E) (%)
100 % RS	159.67	53.22
25 % SMS + 75 % RS	154.13	53.15
50 % SMS + 50 % RS	156.12	55.76
75 % SMS + 25 % RS	150.02	55.56
100 % SMS	149.95	57.67

3.4. The average yield performance and biological efficiency (B.E) of P. ostreatus on different mushroom substrates

Average yield for every flush of *P. ostretaus* grown on SMS with various portions of RS varied significantly as shown in Fig. 2. The highest yields of mushrooms were mostly from the first harvest,

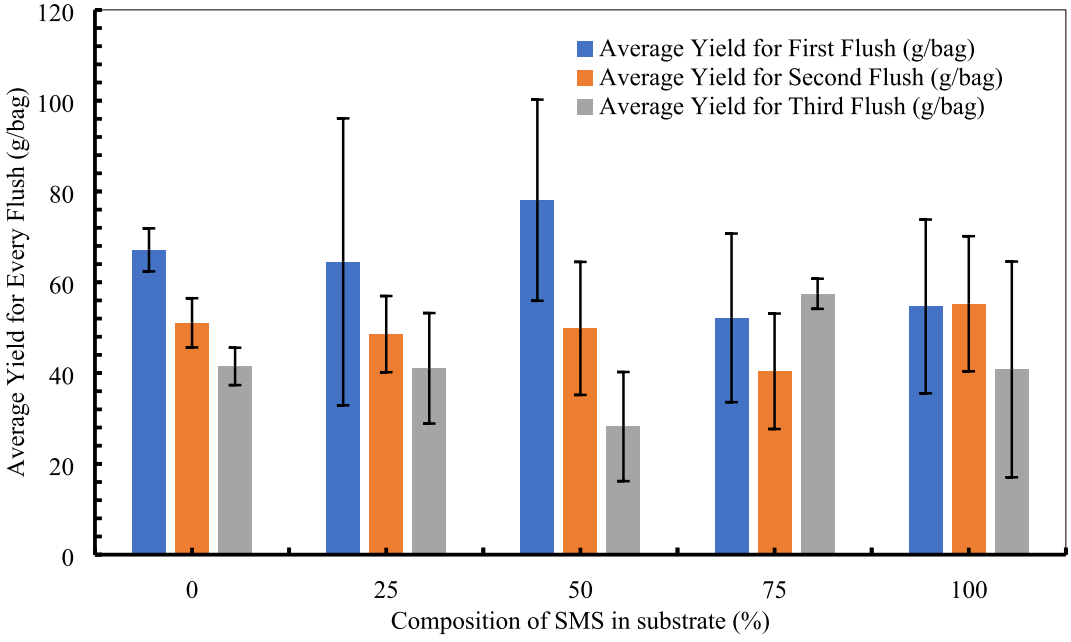

Fig. 2. The average yield performance *Pleurotus ostreatus* for every flush (g/bag.

except those from growing medium 100 % SMS and 75 % SMS + 25 % RS which had the maximum yields from the second flush and third flush, respectively. This could be because of environmental factors like surrounding relative humidity and temperature, and therefore may have affected the first and second flush yield [33]. Average total yield and biological effeciency (B.E) were found to be not significantly different in all formulations as tabulates in Table 6. The Table 6 indicates that the highest average total yield was obtained from 100 % RS substrate (159.67 g/bag), followed by 50 % SMS + 50 % RS (156.12 g/bag), 25 % SMS + 75 % RS (154.13 g/bag), 75 % SMS + 25 % RS (150.02 g/bag) and the lowest total yield was from 100 % SMS (149.95 g/bag) substrate. The average total yield increased when the mushroom substrate had a high portion of RS. This might be due that the mushroom substrate that contains a high content of RS (100 % RS, 25 % SMS + 75 % RS and 50 % SMS + 50 % RS) which had a well-balanced nutrient as compared to the muhsroom substrate that contains a lower portion of the RS. The different content of nitrogen andC/N ratio of mushroom substrate that utilized for growing of the *Pleurotus* spp. affect the performance of yield [23]. Beside, the higher yield performance from the control substrate could be due to the ease of utilization of carbohydrates for the growth of these cellulosic substrates. *Pleurotus*

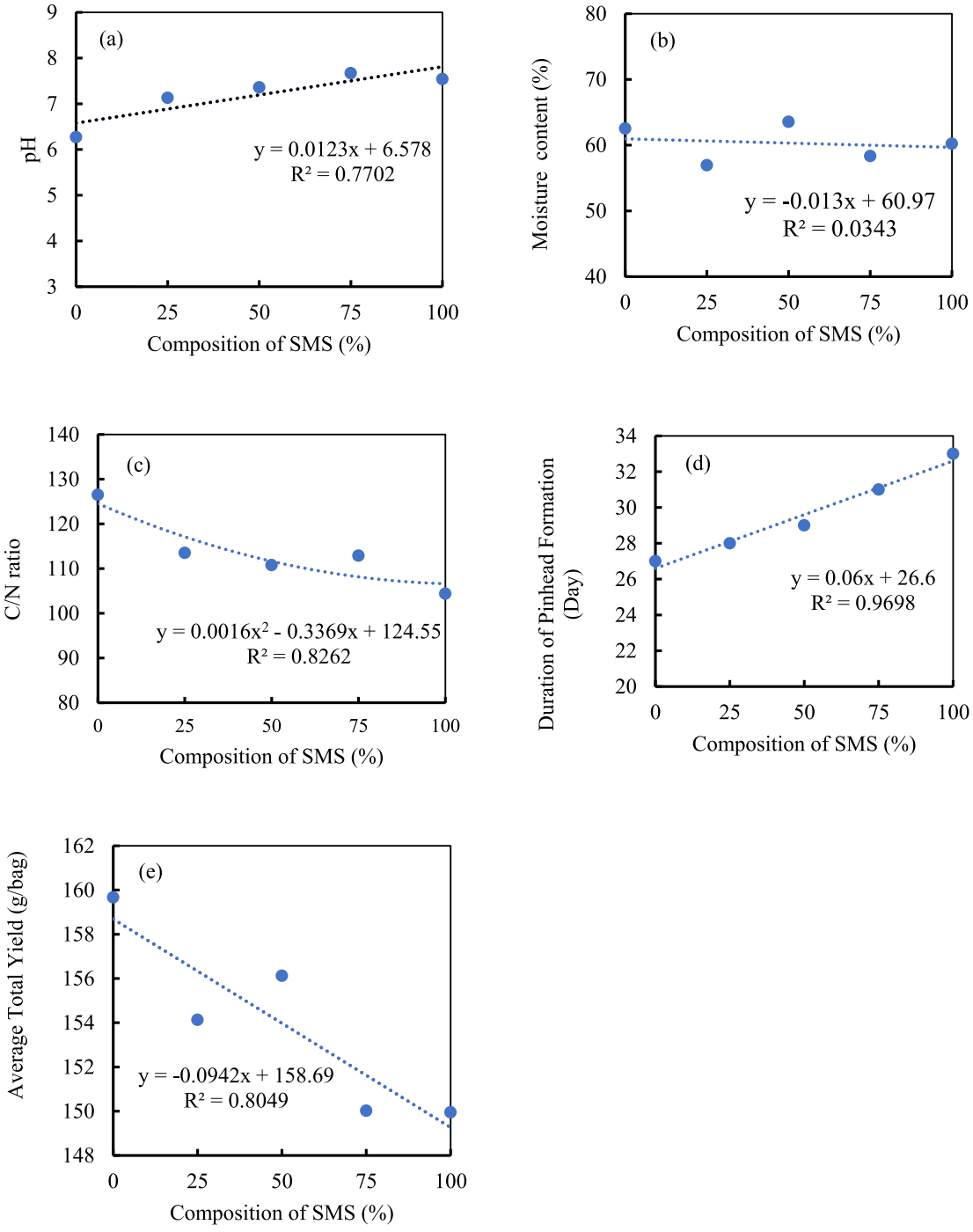

Fig. 3. Growth and yield variable trends of *Pleurotus ostreatus* as the percentage of SMS in the substrate gradually increases: (a) pH fitted to linear model, (b) moisture content fitted to linear model, (c) C/N ratio fitted to quadratic model, (d) duration of pinhead formation fitted to linear model, (e) average total fresh weight fitted to linear model.

spp. grows well on substrates with low nitrogen content of which the C/N ratio is high [23]. Although Khan et al. [24] found the highest yield and mushroom quality was obtained during the first flush, the 100 % SMS and 75 % SMS + 25 % RS mushroom substrate in this study had the highest yield in the second and third flush, respectively. The RS composition which is sawdust was found to consistently be the best substrate for supporting the growth as well as formation of mycelium [34]. In addition, the 100 % SMS had the maximum biological efficiency of 57.98 % even though the 100 % SMS had lower total average yield (149.95 g/bag) followed by 50 % SMS + 50 % RS (55.76 %). However, 25 % SMS + 75 % RS had the lowest biological efficiency (B.E) of 53.15 %. This is consistent with the findings from Jamil et al. [25] where biological efficiency (B.E) values ranged from 17% to 79%. Variation in substrate biological efficiency (B.E) may be attributable to substrate characteristics. Furthermore, it was suggested that differences in the biological effectiveness of different substrates were because of different compositions [26]. It was found that *P. ostreatus* cultivated on 50 % SMS + 50 % RS produced relatively higher average total yield and biological efficiency (B.E), which were 156.12 g/bag and 55.76 %, respectively.

3.5. Regression analysis

Models to represent the relationship of the RS to SMS ratio to various response parameter are shown in Fig. 3. It clearly indicates that linear model can be represented for the effect of average total yield, duration of the pinhead formation, moisture content and the pH. Meanwhile, C/N ratio indicates quadratic relationship towards SMS composition in the substrate. All the model that represent the effect of C/N ratio, duration of the pinhead formation, average total yield and the pH have significant regression correlation, R^2 of 0.82, 0.97, 0.80 and 0.77, respectively. However, insignificant effect of moisture content was observed in this investigation where regression correlation, R^2 is 0.03 (Fig. 3b). It can be seen that insignificant effect of moisture content and pH towards SMS composition was observed in this study because the moisture and the pH were controlled in the range of 58.35–63.55 % and 6.27 to 7.67, respectively.

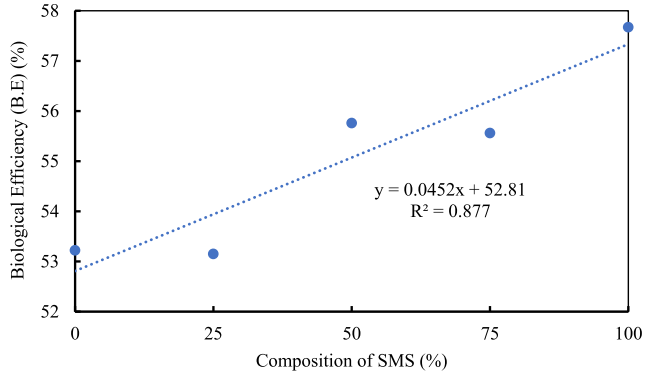

Fig. 4. Biological efficiency (B.E) (%).

The C/N ratio decreased significantly as the SMS composition in the substrate increased, until the minimum C/N ratio of 104.4 was reached at 100 % SMS substrate. The pinhead formation presents a positive linear trend as the composition of the SMS was increased in the substrate (Fig. 3d), while the average total yield of *Pleurotus ostretaus* decreased when the composition of SMS increased in the substrate (Fig. 3e). Biological efficiency (B.E), on the other hands, increased progressively according to a linear model (R^2 = 0.88), where 53.15 % B.E was recorded in 25 % SMS + 75 % RS, and up to 57.67 % B.E in 100 % SMS substrate (Fig. 4). This indicates that differences in terms of mushroom yield and biological efficiency (B.E) are due to the physical and chemical composition of the substrate such as lignin, cellulose, pH, mineral content, C/N ratio and electronic conductivity [14].

3.6. Mushroom mineral composition

P. ostreatus obtained minerals from its mushroom growing substrate. The majority of macroelements in the fresh oyster mushroom were potassium (K), which is followed by sodium (Na), magnesium (Mg) as well as calcium (Ca) as tabulated in Table 7. Minerals are required for water regulation, metabolic responses, and saline balance, rigid formation of bone, sensory stimulation and other function in human [35]. In terms of mineral analysis, fresh mushrooms from the 100 % SMS substrate contained less 96.66 % sodium (Na), less 54.36 % magnesium (Mg), less 44.91 % potassium (K) and less 94.38 % calcium (Ca) than the fresh mushrooms from the control substrate (100 % RS). The minerals content are in line with Heleno et al. [27] where the amount of 615–1093 mg/kg of Na, 391–821 mg/kg of Ca, 1271–1754 mg/kg of Mg and 19496–20388 mg/kg of K were reported. In this study, higher K concentration in the fresh mushroom cultivated from 100 % RS was obtained compared to fresh mushroom from 100% SMS growing substrate which might be due to better absorption of K by *P. ostreatus* fruit bodies from substrate. The ratio between K and Na or (K/Na) in fruits as well as vegetables is commonly more than 2 [28], while pthe K/Na ratio for our mushroom samples were varied from 10.3 to 171.3. From the nutritional point of view, high ratio of K/Na found in oyster mushroom is advantageous and are perfect for individuals with hypertension and cardiovascular disease [29]. Fructiferous bodies have higher Na, Ca and Mg content than other micronutrients, which can be presumed that fresh mushroom in this work has a tendency to gather a greater quantity of these minerals. [30] stated that these distinctions in the mineral components of the fresh oyster mushroom rely on the composition of chemical as well as the substrate's biological nature used for growing. The difference mineral compositions of fresh oyster mushroom can be credited to the accumulation as well as adsorption of these elements from the growing substrate utilized in this research. Table 7 indicates that lower heavy metals were detected for the fresh mushroom *P. ostreatus* which collected for the first time from 100 % RS and 100 % SMS. The Pb, Cd and Cu, which are the main toxins in food, can lead to gradual poisoning. According to WHO/FAO regulation, permitted restriction of lead (Pb), copper (Cu) and cadmium (Cd) are 0.3, 40 and 0.2 mg/kg, respectively [31].

Table 7
Mushroom macroelements of *Pleurotus ostreatus* fruit bodies first harvested from 100% RS and 100% SMS.

Composition of mushroom substrate (%)	Macroelements					Heavy metals		
	mg/kg							
	Na	K	Mg	Ca	K/Na	Cd	Pb	Cu
100 % RS	289.2	2,980.6	133.6	59.3	10.3	0.034	ND*	0.010
100 % SMS	9.7	1,654.5	61.0	3.3	171.3	0.005	ND*	0.006

* ND: Not Detected.

The content of Cu and Cd in this study's mushroom are totally safe to consume.

4. Conclusions

In this present study, the production of *P. ostreatus* from SMS was successfully carried out. There were five compositions of the mixed ratio between rubber tree sawdust and spent mushroom substrates that were analyzed. The results of this study show that there was only a very small difference in terms of growth and yield among five different substrates. The yield of the mushroom was significantly decreased trend as the composition of SMS in the substrate gradually increased while the biological efficiency (B.E) of the mushroom was significantly increased with as low as 25 % SMS in the substrate and showed a significant increasing trend as the composition of SMS in the substrate gradually increased. In addition, it also demonstrates that different substrates have different effects on the productivity and quality of *P. ostreatus* substrates. The usage of SMS as a growth substrate will contribute to their environmentally friendly disposal and simultaneously reduce dependence on the RS. SMS alone and without wheat bran supplementation cannot be used as growing substrate because of its low nutrient. 50 % SMS + 50 % RS with wheat bran supplementation was most suitable alternative substrate to cultivate *P. ostreatus* compared to other substrates.

CRediT authorship contribution statement

Fathie Ahmad Zakill: Conceptualization, Supervision, Validation, Writing - review & editing, Funding acquisition. **Raffizah Mohd Isa:** Formal analysis, Investigation, Writing - original draft. **Mohd Shafiq Mohd Sueb:** Resources, Software, Visualization. **Ruzinah Isha:** Resources, Software, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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