Malaysian Journal of Analytical Sciences
(MJAS)
Published by Malaysian Analytical Sciences Society

PHYSICOCHEMICAL COMPOSITION OF SPENT OYSTER MUSHROOM SUBSTRATE

(Komposisi Fizikokimia bagi Sisa Substrat Cendawan Tiram)

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Received: 8 July 2020; Accepted: 22 September 2020; Published: 10 December 2020

Abstract

Mushroom substrate is a type of lignocellulosic material that helps promote the growth, production, and fruiting of mushrooms. The substrate contains components rich in organic matter due to the modification of the material after harvesting of mushrooms. This study analysed the physicochemical composition of spent oyster mushroom substrate (SOMS) by comparing with sterile fresh mushroom substrate (SFMS). The physicochemical analyses conducted were moisture content, ash content, pH, primary macronutrients (nitrogen, phosphorus, and potassium), secondary macronutrients (calcium and magnesium), micronutrients (iron, manganese, copper, and zinc), and carbon-to-nitrogen (C:N) ratio. The results obtained for moisture content, ash content, pH, and C:N ratio showed higher values for SOMS. The values of moisture, ash content, pH, and C:N ratio increased to 63.00%, 6.58%, 5.92, and 116.29, respectively. For the nutrients in the mushroom substrate, namely phosphorus, calcium, magnesium, iron, and copper, the values after cultivation increased to 57.14 ppm, 7366.67 ppm, 1230.83 ppm, 85.18 ppm, and 3.75 ppm, respectively. Meanwhile, the values of nitrogen, potassium, zinc, and manganese decreased to 0.38%, 706.67 ppm, 16.90 ppm, and 68.65 ppm, respectively. Sulphur content was detected in SFMS but absent in SOMS. In conclusion, mushroom cultivation changed the physicochemical composition of the mushroom substrate.

Keywords: mushroom substrate, comparison, physicochemical analysis

Abstrak

Substrat cendawan merupakan sejenis bahan yang membantu dalam menggalakkan pertumbuhan, pengeluaran dan penghasilan jana buah cendawan. Ia mengandungi komponen yang kaya dengan bahan organik hasil daripada pengubahsuaian kandungan bahan selepas penuaian cendawan. Kajian ini telah menganalisis komposisi fizikokimia sisa substrat cendawan tiram dibandingkan dengan substrat cendawan segar steril. Analisis fizikokimia seperti kelembapan, kandungan abu, pH, makronutrien primer (nitrogen, fosforus, dan kalium), makronutrien sekunder (kalsium dan magnesium), mikronutrien (besi, mangan, tembaga, dan zink), dan nisbah C:N. Keputusan yang diperolehi untuk kelembapan, kandungan abu, pH, dan nisbah C:N menunjukkan nilai yang lebih tinggi untuk sisa substrat cendawan tiram. Peratusan bagi kelembapan meningkat kepada 63.00%, kandungan abu kepada 6.58%, pH kepada 5.92, dan nisbah C:N kepada 116.29. Bagi nutrien dalam sisa substrat cendawan, iaitu fosforus, kalsium, magnesium, besi, dan tembaga, menunjukkan peningkatan selepas penanaman kepada 57.14 ppm, 7366.67 ppm, 1230.83 ppm, 85.18 ppm, dan 3.75 ppm. Bagi nitrogen, kalium, zink, dan mangan, telah menunjukkan penurunan peratusan kepada 0.38%, 706.67 ppm, 16.90 ppm, dan 68.65 ppm. Bagi substrat cendawan segar steril, kandungan sulfat telah dikesan tetapi tidak bagi sisa substrat cendawan. Proses penanaman cendawan telah merubah komposisi fizikokimia dalam substrat cendawan.

Kata kunci: substrat cendawan, perbandingan, analisis fizikokimia

Introduction

The substrate in mushroom cultivation can be defined as a type of lignocellulosic material that promotes the growth, production, and fruiting of mushrooms [1, 2]. Most of the edible species of mushrooms can utilise different types of substrate materials. In Malaysia, the substrate is prepared from rubber sawdust, rice bran, and hydrated lime in the ratio of 100 kg:10 kg:1 kg [3]. Most local farmers in Malaysia typically grow *Pleurotus* species [4] because the processing technology is relatively simple and the materials used in the production of oyster mushrooms are relatively cheap [1, 5]. Also, the species are relatively easy to grow and highly adaptable. Due to their easy preparation, low-cost production technology, and high biological efficiency (BE), the *Pleurotus* species are popular and widely cultivated worldwide, mostly in Asia, America, and Europe [6]. Compared to other mushrooms, the species have a short period of growth [7].

Pleurotus spp. is a saprophyte and it extracts nutrients from the substrate through its mycelium for obtaining essential elements for its growth, such as carbon (C), nitrogen (N), vitamins, and minerals [7]. C and N are the two key macronutrients needed by the fungi for structural and energy requirements. Phosphorus (P), potassium (K), and magnesium (Mg) are also considered as mushroom macronutrients, and trace elements, such as iron (Fe), selenium (Se), zinc (Zn), manganese (Mn), copper (Cu), and molybdenum (Mo), appear to be essential for various functions [8]. In fact, supplementation of the substrate with different materials is advised before spawning to improve the yield of mushrooms [9]. Poultry manure, rice bran, wheat bran, and peat moss have been widely used as food supplements for improved yield, biological efficiency, and growth through the supply of adequate N and slow release of nutrients [10].

Spent mushroom substrate (SMS) refer to the composted material substrate entirely used after many cycles of mushroom cultivation [3, 11]. After several cycles of mushroom cultivation, the nutrients in the substrate decrease and unsuitable to be used for new cultivation [3]. The substrates are an abundant waste product produced by the mushroom industry. For every 1 kg of mushrooms produced, approximately 5 kg of SMS is generated [12, 13]. Respective SMS types have different contents depending on various cultivated mushroom species because the substrates are made from specific ingredients and the preparation method of the substrates, and the form of cultivated mushrooms has different impacts [14]. It is known that different lignocellulose materials can be used as mushroom substrates, particularly for the production of oyster mushrooms, such as rice straw, wheat straw, and sawdust [15].

Thus, this study aims to compare the physicochemical composition of sterile fresh mushroom substrate (SFMS) and spent oyster mushroom substrate (SOMS) in terms of moisture content, ash content, pH value, primary and secondary macronutrients, micronutrients, and carbon-to-nitrogen ratio (C:N ratio) by using t-test analysis as the statistical test.

Materials and Methods

Spent oyster mushroom substrates

The SOMS was obtained from a mushroom grower located at Kampung Empila, Kota Samarahan, Sarawak, Malaysia. Forty five bags of samples were collected randomly after six cycles of harvesting. The SFMS was prepared by mixing softwood sawdust, rice bran, and lime with the ratio of 100:10:1, respectively.

Five bags of SFMS and SOMS were selected. The bags for fresh mushroom substrate were mixed and then three replicates were randomly scooped from the mixture. The same process was applied for SOMS. The substrates were left to dry for a week at room temperature and then shifted through a shifter. The substrates were kept in an airtight container for further analysis.

Physicochemical analysis

Six parameters were considered in this study for physicochemical analysis. The parameters were moisture content, ash content, pH value, primary (N, P, and K) and secondary macronutrients (Ca, Mg, and sulphur (S)), micronutrients (Zn, Fe, Mn, and Cu), and C:N ratio.

Moisture, ash, and pH test

The percentage of moisture was determined by weighing the substrate before and after drying in an oven at 105 °C [13]. The weight loss of the substrate was determined as the moisture content. The ash content was obtained using the standard AOAC method (AOAC, 2000) reported in Rasib et al. [3]. The substrate was incinerated at 550 °C in a furnace overnight. For the pH test, the procedure was based on the methods of Hoa et al. [6]. The substrate was mixed in distilled water with the ratio of 1:10 and the reading was taken by using a pH electrode meter.

Primary and secondary macronutrients and micronutrients

The total C, N, and S of the substrates were determined using a CHNS analyser (Thermo Scientific™ FlashSmart CHNS). Then, the C:N ratio was calculated based on the result of C and N obtained from the analysis.

The determination of P, K, Ca, Mg, Zn, Fe, Mn, and Cu elements was performed using Mehlich 3 (M3) extraction test [16, 17, 18]. This test used inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin Elmer, Optima 8000 ICP) for analysis, except for P. The total P content was determined using an ultraviolet-visible (UV-Vis) spectroscopy (Agilent Cary 60 UV-Vis) at the wavelength of 882 nm.

Statistical analysis

The t-test analysis was performed using the computer software IBM SPSS Statistics version 25 to assess mean significant differences ($p \leq 0.05$) between treatments. The experiments were performed in triplicates.

Results and Discussion

The results of physicochemical properties for SFMS and SOMS are shown in Table 1. The physicochemical composition was characterised after sterilisation for SFMS and after six cycles of mushroom cultivation for SOMS. The raw materials used in SFMS and SOMS were softwood sawdust and rice bran.

SOMS showed higher moisture content that reached 63%, but moisture content dropped significantly to 56.7% for SFMS. Lopez Castro et al. [19] reported that the moisture content for SMS of oyster mushrooms was 46.9%, much lower than the data obtained for SOMS. The result may be due to the difference in the environment where the mushroom grows.

Ash is considered as part of the components for SMS. Table 1 shows the results of ash content for SFMS that reached 4.16% and increased significantly to 6.58% for SOMS. There was an increase in the amount of ash after some time. The increasing amount of ash in the SMS showed the number of extractives in SOMS after many processes involved, such as sterilisation and several cycles of mushroom cultivation [3]. According to Rasib et al. [3], the ash content for mushroom substrate was 4.345% and 5.299% for the SMS of oyster mushrooms.

From the determination of hydrogen ion concentration (pH), the substrate was acidic at an average pH of 5.85. A higher pH was recorded for SOMS (5.92), whereas for SFMS, the pH was 5.78. Based on the study of Sultana et al. [20], the optimum pH range for mycelium growth was estimated at 5.5-6.5 for SFMS. Therefore, the result for SFMS is within the optimum range for mushroom growth. For the SMS of oyster mushrooms, the optimum range was 5.1-7.4, as stated by Paredes et al. [21] and the study by Sendi et al. [22] recorded the pH value of 6.10, which was still within the range.

The results of the C content of the substrates are shown in Table 1, which reported the influence of cellulosic composition before and after mushroom cultivation on the C level. Higher C content was detected in SFMS at 45.22% before mushroom cultivation, and the C content declined significantly to 43.87% for SOMS at the end of mushroom cultivation. From the results, it showed that SOMS had lower C content than the results of the substrate before oyster mushroom cultivation. The low C content is linked with the release of carbon dioxide (CO_2) by fungal exoenzymes during development process [23]. This phenomenon is due to the growth of mushroom mycelium during cultivation that contributed to the decomposition of cellulosic matter and released CO_2, thus leading to low C content.

The C:N ratio is very significant based on their role in the growth of mushrooms. Based on the results, SOMS achieved a higher C:N ratio (116.29%) than SFMS (102.69%). The C:N ratio increased in SOMS after six cycles of harvesting. The finding is expected due to the decrease of total C and N in SOMS because mushrooms consume C and N for growth. Also, the C:N ratio was higher than other studies due to the materials used in the substrate. For example, the substrate produced from wheat straw contained 38.50 of C:N ratio [23]. The only supplement added in the substrate was rice bran. Other substrates used other types of supplements that may differ in chemical composition. Thus, the value of C:N ratio may differ from other substrates. Furthermore, the C:N ratio of sawdust or woody tissues is 350:1 to 500:1. Thus, wood-inhabiting mushrooms, such as the *Pleurotus* species (oyster mushroom), have a unique ability to grow in such substrates, which suggests that these mushrooms can metabolise large amounts of carbohydrates, including lignin, in the presence of a very small amount of N [1].

The substrate directly affects the mineral composition as the hyphae of the fungi are in contact with the compound and withdraw essential elements [7]. The primary macronutrients for substrate, including N, P, and K, showed significant results for both SFMS and SOMS. As tabulated in Table 1, a higher N content was achieved for SFMS (0.44%) than SOMS (0.38%). The P content for SFMS was 10.73 ppm, which then increased significantly to 57.14 ppm for SOMS. The K content was 1,634.17 ppm for SFMS and decreased to 706.67 ppm for SOMS. The values of N and K decreased as mushrooms used the nutrients for growth. As for P, before cultivation, the amount of P was low because lignocellulosic materials are usually low in mineral content [7,24] and after cultivation, sawdust-based SMS contained high P content [25] and SOMS was made of sawdust-based substrate. The study from Sendi et al. [22] showed that the value of N in SMS was 0.34% compared to SOMS (0.38%), which only had a small difference. The values of P and K for SMS were 0.16% and 0.53%, respectively [22], and 0.006% of P and 0.07% of K for SOMS. The difference may be due to the raw materials used in making substrates for both studies.

The secondary macronutrients, including Ca, Mg, and S, were tested for SFMS and SOMS. SOMS showed higher Ca content (7366.67 ppm) than SFMS (3046.67 ppm). Meanwhile, the Mg content of SFMS was lower (525.83 ppm) than SOMS (1230.83 ppm). The total S was only detected in SFMS (0.16%) and for SOMS, the total S was below the detection limit based on the analysis from the CHNS analyser. According to Sendi et al. [22], the values of Ca and Mg detected for SMS were 0.51% and 0.15%, respectively. Compared to the data obtained for SOMS from this study, the values in percentage of Ca are 0.74% and 0.12% for Mg.

The micronutrients for both SFMS and SOMS, which include Zn, Mn, Fe, and Cu, were tested. The Zn content for SFMS was higher (77.50 ppm) than SOMS (16.90 ppm). For the Mn content, the value of SOMS was lower (68.65 ppm) than SFMS (97.50 ppm). The Fe content for SFMS was 20.00 ppm and then increased significantly to 85.15 ppm for SOMS. The Cu content increased significantly from SFMS (0.72 ppm) to SOMS (3.75 ppm). From the results obtained, the total Zn and Mn for SOMS decreased from SFMS, whereas for the total Fe and Cu, there are differences in the values for SOMS, which increased from SFMS. In the study by Medina et al. [26], the Fe content for SOMS was 337 mg/kg, significantly higher than the data obtained for SOMS at 85.15 ppm in this study. The Cu, Mn, and Zn contents at the values of 5.5 mg/kg, 49 mg/kg, and 20 mg/kg, respectively, are still within the range with the results in Table 1.

Table 1. Sterile fresh mushroom substrate and spent oyster mushroom substrate

Parameters	**Sterile Fresh Mushroom Substrate (SFMS)**	**Spent Oyster Mushroom Substrate (SOMS)**	**LSD ($p \leq 0.05$)**
pH	5.78 ± 0.03	5.92 ± 0.02	0.002
Carbon: Nitrogen Ratio	102.69 ± 4.80	116.29 ± 1.42	0.009
	------------------------ %	------------------------	
Moisture	56.70 ± 0.10	63.00 ± 0.78	0.005
Ash	4.16 ± 0.01	6.58 ± 0.02	< 0.001
Total Carbon	45.22 ± 0.26	43.87 ± 0.25	0.003
Total Nitrogen	0.44 ± 0.02	0.38 ± <0.01	0.009
Total Sulphur	0.16 ± 0.04	Null	-
	----------------------- ppm	----------------------	
Phosphorus	10.73 ± 0.74	57.14 ± 3.58	0.001
Potassium	1634.17 ± 72.86	706.67 ± 118.75	< 0.001
Calcium	3046.67 ± 178.79	7366.67 ± 580.04	< 0.001
Magnesium	525.83 ± 20.82	1230.83 ± 100.38	< 0.001
Zinc	77.50 ± 4.33	16.90 ± 0.58	0.001
Manganese	97.50 ± 4.33	68.65 ± 2.21	0.001
Iron	20.00 ± 6.61	85.18 ± 2.43	< 0.001
Copper	0.72 ± 0.03	3.75 ± 0.13	< 0.001

Conclusion

There is a significant difference between SFMS and SOMS in terms of physicochemical analysis. From the results obtained, in terms of moisture content, pH, and ash content, there are significant differences between both substrates. The data obtained showed increased moisture, pH, and ash content after mushroom cultivation. For primary macronutrients (N, P, and K), there is a significant difference between the substrates. The values of N and K decreased after six cycles of mushroom cultivation as mushrooms needed N and K for growth. For secondary macronutrients, Ca and Mg increased and are significantly different, whereas S was only detected in SOMS. Lastly, for micronutrients, the values of Fe and Cu increased, but the values of Zn and Mn decreased after mushroom cultivation. Thus, this study is valuable for future reference as the valuable nutrients in SOMS after mushroom cultivation can be used for further usage in the agricultural field and others, instead of throwing away the substrate.

Acknowledgement

The authors would like to thank the Tun Openg Chair Grants for the support through grant (F07/TOC/1739/2018), the Faculty of Resource Science and Technology for the research facilities provided and to Mr. Azis Ajim, the mushroom grower, for providing the spent oyster mushroom substrates for this study.

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