

## CHARACTERIZATION OF BIOCHEMICAL COMPOSITION FOR DIFFERENT TYPES OF SPENT MUSHROOM SUBSTRATE IN MALAYSIA

(Pencirian bagi Komposisi Biokimia bagi Pelbagai Jenis Sisa Substrat Cendawan di Malaysia)

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### Abstract

A preliminary study was conducted to identify the amount and changes of biochemical composition of different types of Malaysian spent mushroom substrate (SMS) before and after several cycle of mushroom cultivation. The characterization of SMS involved the analysis of crude protein, carbohydrate, fat, lignin and ash for selected mushrooms namely as white oyster (*Pleurotus ostreatus*), grey oyster (*Pleurotus sajor-caju*), abalone (*Pleurotus cystidiosus*), ganoderma (*Ganoderma lucidium*) and black jelly (*Auricularia polytricha*). Overall trend showed that there were increment for crude protein and fat, whereas carbohydrate and lignin showed reduction in the content. Significant results were showed on protein increment where ganoderma attained the highest value, 36.6 g, followed by black jelly, white oyster, grey oyster and abalone. Contradictory, lowest carbohydrate reduction was observed in ganoderma at 70.42 g and the most was in black jelly. Increment in fat and reduction in lignin was almost similar for each SMS. There was an increment in the ash percentage resulted from sterilization process. Clearly cultivation by mushroom had changed biochemical value especially in increasing the protein content that might be useful in protein required industry such as animal feeding.

**Keywords:** Spent Mushroom Substrate (SMS), biomass, biochemical composition

### Abstrak

Satu kajian awal telah dijalankan untuk mengenalpasti jumlah dan perubahan komposisi biokimia pelbagai jenis sisa substrat Cendawan (SMS) di Malaysia sebelum dan selepas beberapa kitaran tuaian cendawan. Pengkriteriaan SMS melibatkan analisis seperti protein mentah, karbohidrat, lemak, lignin dan abu untuk cendawan yang terpilih seperti tiram putih (*Pleurotus ostreatus*), tiram kelabu (*Pleurotus sajor-caju*), abalon (*Pleurotus cystidiosus*), ganoderma (*Ganoderma lucidium*) and telinga kera (*Auricularia polytricha*). Tren keseluruhan menunjukkan terdapat peningkatan untuk protein mentah dan lemak manakala karbohidrat dan lignin menunjukkan penurunan di dalam kandungan. Keputusan yang signifikasi telah ditunjukkan di dalam peningkatan protein di mana ganoderma mencapai jumlah tertinggi, 26.6 g diikuti cendawan telinga kera, tiram putih, tiram kelabu dan abalon. Sebaliknya, jumlah penurunan karbohidrat yang terendah direkodkan di dalam ganoderma sebanyak 70.4 g dan kebanyakannya di dalam cendawan telinga kera. Peningkatan lemak dan penurunan di dalam lignin menunjukkan keputusan yang hampir sama bagi setiap SMS. Terdapat peningkatan peratus abu yang disebabkan daripada proses steril. Jelas menunjukkan bahawa proses tuaian oleh cendawan telah mengakibatkan perubahan daripada segi komposisi biokimia terutamanya di dalam peningkatan jumlah protein yang mungkin berguna di dalam industri yang memerlukan protein seperti makanan haiwan.

**Kata kunci:** Sisa Substrat Cendawan (SMS), biojisim, komposisi biokimia

### Introduction

Abundant of organic wastes are being produced nowadays. Majority of the waste could be converted into beneficial product through appropriate bioconversion technology. Spent Mushroom Substrate (SMS) is one of the organic wastes which are now been given attention for spent utilization after mushroom cultivation process. SMS refer to

the composted material substrates that have been fully utilized after several cycle of harvesting mushroom cultivation which contain high organic matter [1]. This waste material could be enriched with extracellular enzyme such as lignin peroxidase, laccase and manganese-dependent peroxidase and other microorganism after several series cultivation of mushroom [2] and contains relatively high levels of nitrogen, potassium, phosphorus, calcium and trace element notably silicon and iron [3,4].

In Malaysia, the compost was initially manufactured from raw material contained of rubber saw dust, rice bran and hydrated lime in ratio of 100kg: 10kg: 1kg respectively before being induced by different types of mushroom spawn such as white oyster (*Pleurotus ostreatus*), grey oyster (*Pleurotus sajor-caju*), abalone (*Pleurotus cystidiosus*), ganoderma (*Ganoderma lucidium*) and black jelly (*Auricularia polytricha*) [5]. The rubber saw dust was used as a base medium for composting as Malaysia is a rich with rubber plantation country. After several cycle of mushroom cultivation, SMS basically will be openly burning as it could not be reused for the next cultivation due to preservation quality of mushroom itself [6]. Others disposal strategy including burying, composting with animal manure or landfilling can also be applied whenever possible. Generally, mycelium takes about 60 days to fully colonize the bag of substrate and followed growing of the mushroom bodies afterward. Each cycle take one or two weeks to produce the fruiting bodies depended to temperature, relative humidity, pH and light requirement

Amount of accumulated SMS is resulted from the volume of mushroom production. Approximately, production of 1 kg mushroom will generate 5 kg of SMS [7]. In Malaysia, production of mushroom per month is about 8,424 metric tons and consequently, SMS accumulation is amounted to 42,120 metric tons per month [8]. That amount of SMS give challenge for disposal management and therefore an effective management is needed. One of the solutions is to utilize those SMS and there are potential in sectors such as enzyme production, animal feeding and land amendment. This intention was in line with the National Agro food Policy (2011-2020) which focuses on increasing mushroom production and in the same time to explore the use of agro waste into beneficial product [9]. Before doing so, characterization of the SMS content is a crucial task to be considered.

Thus, the aim of this study is to characterize nutritional values including protein, carbohydrate, fat, lignin and ash content for different types of SMS such as white oyster, grey oyster, abalone, ganoderma and black jelly from initial stage (fresh medium), second stage (sterile fresh medium) and final stage which is after several time of mushroom cultivation for any future application.

#### Materials and Methods

Triplicate bags of SMS were selected randomly from seven types of SMS from Ganofarm Sdn. Bhd, Tanjung Sepat, Selangor. In total, twenty seven bags were used in this study. The sample was immediately dried at 50°C for 24 hours before being ground with Waring commercial lab blender and sieved at 550µm particle size. Then, the SMS was separated according to its type and kept in an airtight container for further analysis. All chemicals used are analytical grade. Table 1 show the list of SMS used stage and biochemical composition test conducted.

For sampling and analysis method, 100mg of sample was taken from each container respectively to their types for determination of crude protein value. For the final stage, only group of ganoderma were taken after 2 times of cultivation cycle compared to another types – (black jelly, white oyster, grey oyster and abalone) 7 times due to preservation quality of ganoderma itself. The protein was analyzed using modified Lowry method [10]. All experiment and chemical analysis were performed in triplicate and results were demonstrated in mean  $\pm$  SD calculated by using Microsoft Excel (Version 14.0). Significant difference tests were performed by using *t*-test from Excel 14.0 to determine protein and carbohydrate value. Carbohydrate was analyzed using Phenol-sulphuric method [11], fat using soxtec method [12], lignin using extraction and hydrolysis method [13] and ash content using standard AOAC method [14].

Table 1. Types of SMS and biochemical composition analysis performed in this study

| Stage   | Types of substrate                  | Cycle of cultivation | Biochemical composition analysis |
|---------|-------------------------------------|----------------------|----------------------------------|
| Initial | Fresh Medium (FM)                   | none                 |                                  |
| Second  | Sterile Fresh Medium (SFM)          | none                 | Protein<br>Carbohydrate          |
| Final   | Abalone ( <i>P.cystidiosus</i> )    | 7 times              | Fat                              |
|         | Black Jelly ( <i>A.polytricha</i> ) | 7 times              | Ash                              |
|         | Grey Oyster ( <i>P.sajor-caju</i> ) | 7 times              | Lignin                           |
|         | White Oyster ( <i>P.ostreatus</i> ) | 7 times              |                                  |
|         | Ganoderma ( <i>G.lucidum</i> )      | 2 times              |                                  |

### Results and Discussion

Protein, carbohydrate, fat, lignin and ash are part of the components of SMS. Protein in substrate serves as nitrogen source for development of mushroom fruit bodies whereas lignin are one of the main source of carbon and energy for the fruiting bodies in order to developed. Their utilization and degradation can be greatly influence the mushroom bodies to growth. The biochemical composition of SMS were characterized at initial stage (fresh medium) which just after the mixture of compost substrate were mixed altogether, second stage (rubber sawdust) when mixed compost substrate had gone for sterilization process at 100° C for 8 hour and lastly final stage (SMS) after several cycle of mushroom cultivation depend to types of mushroom spawn. Overall results on biochemical composition analysis for different types of Spent Mushroom Substrate (SMS) are shown in Table 2.

Table 2. Biochemical composition analysis for different types of Spent Mushroom Substrate (SMS)

| Stage   | Types of substrate                   | Cycle of cultivation | Protein Yield (g/kg) | Carbohydrate yield (g/kg) | Fat Lignin yield (g/kg)     | Ash (%)      |
|---------|--------------------------------------|----------------------|----------------------|---------------------------|-----------------------------|--------------|
| Initial | Fresh medium (FM)                    | -                    | 0.54± 0.01           | 81.13 ± 0.01              | 10.08± 0.02<br>90.73 ±0.02  | 1.285± 0.002 |
| Second  | Sterile Fresh Medium (SFM)           | -                    | 0.66±0.02            | 81.06 ±0.02               | 10.16± 0.02<br>90.60 ± 0.21 | 4.345±0.002  |
| Final   | Black Jelly ( <i>A.polytricha</i> )  | 6 - 7                | 19.3± 0.26           | 57.49±0.02                | 22.89±0.04<br>71.07±0.02    | 5.867± 0.002 |
|         | Grey (P.S.C) ( <i>P.sajor-caju</i> ) | 6 - 7                | 14.5± 0.32           | 61.45±0.03                | 23.22±0.03<br>70.27±0.02    | 5.146±0.002  |
|         | White ( <i>P.ostreatus</i> )         | 6 - 7                | 16.1± 0.32           | 63.57±0.02                | 23.78±0.04<br>70.67±0.06    | 5.299±0.011  |
|         | Ganoderma ( <i>G.lucidum</i> )       | 2                    | 36.6± 0.21           | 70.42±0.04                | 25.56±0.03<br>72.13±0.02    | 5.605± 0.002 |

From Table 2, the results show that crude protein value had increased more than twice from initial stage of fresh medium 0.54 g till average 15.95 g for group (black jelly, white oyster, grey oyster and abalone) whereas protein content in ganoderma had shown the great increment which is 36.6 (g/kg) due to mycelium present in SMS. Types of substrate contribute to the amount of the protein and fat in SMS. The main ingredient, the rubber sawdust, was found high in nitrogen content that known to promote mycelium development and fruiting bodies formation in mushroom grow. Thus, after certain period of cultivation process, nitrogen helps develop the mycelium which covering and colonizing the whole substrate and as a result, the protein and fat level had increased from initial stage till several cycle of cultivation. This result was similar and comparable to the findings by Peredes et al. (2009) [15]. The crude protein and fat content was estimated using Equation (1) and (2) respectively.

$$Y = mX + C \quad (1)$$

where Y is absorption of sample at 660nm (A) from UV-vis spectrometer, m is gradient value and C is y- intercept. Both of m and C value are automatically generated while plotting the standard calibration curve. Whereas in Equation (2), W is weight of extraction cup with fat residue (g), W1 is weight of empty extraction cup (g) and W2 is the weight of sample (g).

$$\text{Fat (\%)} = \frac{W - W_1}{W_2} \times 100 \% \quad (2)$$

Fungi are capable to degrade lignocellulosic materials due to highly efficient enzymatic system [16]. Fungi specifically mycelium have two types of extracellular enzymatic system such as hydrolytic system that producing hydrolases (lignin peroxidase, laccase and manganese-dependent peroxidase) which responsible for polysaccharide degradation and use them as nutrient for growth and fructification after being induced by spawn in the beginning of mushroom production process [17]. Others, oxidative and extracellular ligninolytic system degrade lignin and open phenyl rings in SMS. It has prove that the enzymes from mycelium helps to breaks down the lignin in substrate in order to get the carbon source which is cellulose in order to grow the mushroom and making the lignin value of substrate become increase within the time.

Whereas, for cellulose value become less within the time because it has being used as carbon source or energy for the fruiting bodies of mushroom to developed. In this case, as the production of mushroom is continuous process till the maximum yield of cultivation around 7 to 8 times achieved has result the degradation of carbohydrate and lignin. The carbohydrate amount decrease from initial stage of fresh medium 81.13 (g/kg) to lowest yield of carbohydrate which is in black jelly 57.49 (g/kg) whereas lignin decrease from initial stage of fresh medium 90.73 (g/kg) till the lowest yield of 70.27 (g/kg) in grey oyster. All the carbohydrate and lignin content were estimated using Equation (3), (4) and (5) respectively.

$$Y = \frac{X}{0.1} \times 100 \quad (3)$$

X is absorbance corresponds to amount of carbohydrate present in solution. For Equation (4) and (5), L1 is amount of lignin present in sample, W1 is initial weight of sample after furnace process, G4 is weight of sample after going through hydrolyze and boiling process and G3 is weight of sample after extraction process.

$$L1 = \frac{(1 - W_1)G_4}{G_3} \quad (4)$$

$$\text{Lignin (\%)} = \frac{L1}{15} \times 100 \% \quad (5)$$

The increasing amount of ash in SMS indicate the amount of extractive in SMS itself after several process involved such as sterilization process in second stage and several cycle of mushroom cultivation process. The amount of ash content in SMS increase from 1.285 % till maximum yield 5.605 % in *G.lucidum*. The ash content was estimated using Equation (6).

$$\text{Ash (\%)} = \frac{M_3 - M_1}{M_2 - M_1} \times 100 \% \quad (6)$$

### Conclusion

The cultivation of different types of mushroom on rubber sawdust had changed the biochemical composition of protein, carbohydrate, fat, lignin and ash throughout mushroom growing period. Spent Mushroom Substrate (SMS) that induced by *Ganoderma (G.lucidum)* showing more compromising compositional biochemical value compared to other types (black jelly, white oyster, grey oyster and abalone) which crude protein content showing great increment compared to the others. Utilization of carbohydrate in SMS in order to grow the mushroom makes the carbohydrate content become decrease over the time. This finding could provide information for further applications on SMS in industry such in field of enzyme production, animal feeding, bioremediation and others in order to manage the biomass effective and efficiently in future.

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