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NUTRITIONAL COMPOSITION OF *Volvariella volvacea* GROW USING DIFFERENT CULTIVATION TECHNIQUES AND SUBSTRATE UTILIZATION

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Abstract

Volvariella volvacea is a nutritious edible mushroom that is found and cultivated mainly in Southeast Asian countries. The nutritional composition and energy value of *V. volvacea* are analyzed using proximate analysis. The proximate analysis is useful for assessing its potential health benefits and nutritional value. However, different cultivation methods and substrates may produce different proximate compositions. The cultivation method may influence several factors, such as the substrate, the environmental conditions, the duration of cultivation, and the presence of any additives or fertilizers. This study aims to compare the proximate analysis of *V. volvacea* grown under different cultivation techniques with varying substrate utilization. The data were compared to findings from this study and the previous report that demonstrated a comparable parameter. Proximate analysis for this study was conducted to determine the moisture, carbohydrate, protein, fat, fiber, and ash content using indoor cultivation techniques and POEFB fiber pellet as a substrate. Results showed the mushrooms produced in this study have a moisture (86%), carbohydrate (8.9%), protein (4.0%), crude fat (0.1%), fiber (0.3%), and ash content (1.0%). Data comparisons with previous studies revealed that indoor cultivation yielded a lower protein content compared to the wild cultivation method. Interestingly, *V. volvacea* grown on POEFB fiber produced a high carbohydrate content compared to the other substrates (paddy straw, cotton waste, and banana leaves). It was also found that other components such as moisture, protein, fat, and ash showed a lower percentage when cultivated using a substrate other than POEFB. In conclusion, it was suggested that different cultivation methods and substrate utilization result in variations in proximate analysis. This could be due to differences in the substrate's nutrient availability and composition. In the future, research on the productivity of *V. volvacea* grown under different growth conditions is strongly recommended.

Keywords: mushroom cultivation, palm oil empty fruit bunch fiber, proximate analysis, *Volvariella volvacea*

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Introduction

Volvariella volvacea is one of the edible mushrooms found in countries with tropical weather. Several Southeast Asian countries, including India, Thailand, the Philippines, Indonesia, and Malaysia, have cultivated it since its first discovery in China (Jemsi & Aryantha, 2017; Rosmiza et al., 2016; Biswas & Layak, 2014). It is also known as a paddy straw mushroom. Today, farmers also cultivate it using alternative materials like banana leaf waste, cotton waste, bamboo waste, water hyacinth, and palm oil empty fruit bunch (POEFB) (Biswas, 2014; Thiribuvanamala et al., 2012). Due to its short cropping period, farmers prefer to cultivate *V. volvacea* and sell it to consumers (Thuc et al., 2020). People typically add this mushroom to their daily meals or deep-fry it for snacking. It is wildly popular because of its high nutritional and medicinal properties, which are attributed to its high carbohydrate, protein, and fiber content (Kupradit et al., 2023). Another intriguing feature of *V. volvacea* is that its texture is like that of poultry meat. This makes the mushroom the most suitable choice for protein alternatives in

vegan or vegetarian foods. Like other cultivable edible mushrooms, *V. volvacea* has a high nutritional and medicinal content. Even though *V. volvacea* has a lot of benefits, cultivation using POEFB pellets is rather unfamiliar in the industry. Previous research has solely utilized the raw and original form of POEFB, which is notably larger. This has caused several problems, including difficulty storing and transporting the bunches. Therefore, this paper introduces POEFB fiber pellets as an alternative to POEFB bunches in *V. volvacea* cultivation. However, due to the proposed alternative cultivation methods, there is a gap in understanding how different cultivation methods and substrates impact its nutritional composition. Proximate analysis provides a detailed profile of these nutritional components and is critical for assessing the mushroom's potential health benefits and nutritional value.

This research aims to conduct a proximate analysis of *V. volvacea* grown on different substrates to determine how cultivation practices influence its nutritional composition. By identifying the optimal growing conditions, this study seeks to enhance the nutritional value of *V. volvacea*, thereby contributing to better dietary options and agricultural practices. Proximate analysis is commonly used to quantify and analyze the nutritional content present in the *V. volvacea* fruiting body (Singh & Singh, 2023). Therefore, we can use the study's findings to forecast the effectiveness of POEFB as a substrate for *V. volvacea* cultivation and to guide future research on this species. This paper focused on explaining the results obtained from the proximate analysis and was compared with previous findings for validation.

Methods

Materials and Methods

Volvariella volvacea was cultivated on POEFB pellets using indoor cultivation methods. Their growth is monitored and recorded daily. Once the mushroom reaches the egg stage, it is harvested and transferred to the lab for further analysis. The proximate analysis was conducted based on six parameters, which are the determination of carbohydrate, protein, crude fat, crude fiber, moisture content, and ash. The methods for proximate analysis were employed and adapted from Badhai (2023). Data comparison was done using the data retrieved from previous research reports using wild cultivation on POEFB (Masitah et al., 2023) and indoor cultivation using different substrates (Zikriyani et al., 2018).

Determination of Carbohydrate

The carbohydrate percentage is the result of a simple calculation formula, as described by Zhou et al. (2019). The formula was stated as below:

Total carbohydrate (%) = 100 - (% Ash + % Moisture + % Protein + % Fat)

Determination of Protein

The protein content determination of the *V. volvacea* sample was conducted by implementing the basic principle of the Kjeldahl method as described by Jensen and Cottrell (2020). The first step is to digest the sample by grounding it into a fine powder using a laboratory mill to ensure uniformity. A total of 5 g of *V. volvacea* sample was weighed into a digestion flask containing 5 ml of sulfuric acid and 2 g of sodium sulfate. Then, 2 g of copper sulfate was also added as a catalyst. The flask was heated at 50 ºC for 2 hours. The digestion process converts organic nitrogen into ammonium sulfate. The next step is neutralization, in which the digested solution is neutralized using sodium hydroxide to convert ammonium sulfate into ammonia gas (NH_3). Then, NH_3 gas was distilled off and absorbed in boric acid. The last step is titration, in which the amount of ammonia absorbed in the hydrochloric acid was determined by back-titration. The endpoint was indicated when the colour of the solution turned yellow. The blank test using 1 g of saccharose was done at the same time as the sample analysis.

All the formulas used in protein content determination are listed below:

Nitrogen content (%) = $V_a - V_b \times 1.4007\ W \times 100$

where:
V_a =titration volume of sodium hydroxide for sample
V_b = titration volume of sodium hydroxide for blank
W = weight of the sample

The nitrogen content was converted into crude protein using protein factor 6.25 (the conversion factor), which is typically used for most food products to convert nitrogen content to protein content:

Crude protein (%) = Nitrogen content (%) × 6.25

Determination of Crude Fat
The crude fat content analysis was done using the Soxhlet apparatus according to the Soxhlet method. A total of 5 g of sample was added to the thimble and dried in the oven at 102 °C for 5 hours. The thimble was then inserted into the soxhlet extractor. A round-bottomed flask containing 90 mL of petroleum ether was fitted at the bottom of the Soxhlet extractor. The extraction unit was assembled over a water bath in a fume hood. The flask was heated to the boiling point. The extraction was continued for 6 hours. The flask was removed from the unit, and the remaining contents were further dried in the oven at 102 °C for 2 hours. The flask was cooled down in the desiccator. The weight of the flask with the remaining content was measured and recorded as the final weight. The crude fat content was then calculated as follows:

$$\text{Crude fat (\%)} = (\text{Final Weight (g)} - \text{Weight of empty flask (g)}) \times \frac{100}{\text{Weight of sample (g)}}$$

Determination of Crude Fiber
The weight of the sample was measured and recorded as the initial weight. A total of 200 ml of 5% hydrochloric acid was added to the sample in a beaker. The solution was further heated in a 90 °C water bath for 2 hours. The solution was cooled down and filtered through the filter paper. The filtrate was kept in a beaker and added to 200 ml of sodium hydroxide. The solution was heated again at 90 °C for 2 hours. The solution was filtered again using filter paper. The filtrate was washed with hot water, followed by acetone. The filtrate was further dried in the oven at 120 °C for another 2 hours. The weight of the filtrate was measured and recorded as the final weight. The percentage of weight loss in the sample was determined as the percentage of crude fiber.

Determination of Moisture Content
The harvested *V. volvacea* egg was cut into slices as preparation for moisture content determination. The total weight of the *V. volvacea* slices was recorded as the initial weight. The *V. volvacea* slices were then dried overnight for 4 hours at 60 °C. The dried *V. volvacea* slices were weighed again and recorded as the final weight. The moisture content was then calculated using the formula below:

$$\text{Moisture (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

Determination of Ash
The *V. volvacea* sample that had been dried previously was used to determine the ash content. The weight of the dried sample was recorded as the initial weight. The samples were transferred into crucibles and placed in a furnace. The samples were heated at 500 °C for 4 hours. After four hours, the crucible was transferred into a desiccator and cooled down to room temperature. The weight of the sample was measured again and recorded as the final weight. The ash content was calculated following the formula below:

$$\text{Ash (\%)} = \frac{\text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

Determination of Energy

The crude fat content was determined using a diethyl ether solvent to extract the dry materials from *V. volvacea*. The solvent was then removed by using a rotator evaporator, and the weight of the dried sample was measured. The crude fat content is then calculated using the following formula:

$$\text{Crude Fat (\%)} = \frac{\text{Weight of ether extract (g)}}{\text{Weight of dried sample (g)}} \times 100$$

Result and Discussion

Proximate analysis of *Volvariella volvacea* cultivated on different types of substrates

Table 1 represents the proximate compositions (g/100 g) (on a dry weight basis) of *V. volvacea* grown using different cultivation strategies and types of substrates used during cultivation. The data comparison clearly demonstrated that indoor cultivation led to a lower composition in most tested components compared to wild-cultivated *V. volvacea*. This result might be attributed to a different environmental environment factor that captures the productivity and results of the nutrient composition produced by the mushroom. In wild cultivation, other constituents or variable factors may interfere with or influence the production of nutrient content. As in indoor cultivation, the environmental and other parameters were set in a controlled environment.

Table 1. Data comparison of proximate analysis of *V. volvacea* cultivated using different cultivation techniques and sources of substrate

Composition (%)	Indoor Cultivation				Wild cultivation
	POEFB fiber (This study)	Paddy Straw (Zikriyani et al., 2018)	Cotton Waste (Zikriyani et al., 2018)	Banana Leaves (Zikriyani et al., 2018)	Raw POEFB (Masitah et al., 2023)
Crude fat	0.1	0.0	0.07 ± 0.12	0.12 ± 0.16	2.43 ± 0.14
Protein	4.0	4.69 ± 1.03	3.51 ± 0.40	2.62 ± 0.16	20.73 ± 1.16
Carbohydrate	8.9	3.74 ± 0.37	4.26 ± 0.84	5.11 ± 0.21	8.78 ± 0.17
Fiber	0.3	NA	NA	NA	NA
Ash	1.0	1.08 ± 0.03	0.92 ± 0.07	0.76 ± 0.04	6.57 ± 0.01
Moisture	86.0	90.49 ± 1.43	91.25 ± 0.85	91.40 ± 0.17	71.47 ± 1.22

Crude fat content in indoor cultivated *V. volvacea* was found to be low in content compared to the wild cultivated mushroom. These results agreed with earlier studies by Manjunathan & Kaviyarasan (2011), which demonstrated that *V. volvacea* has a low-fat concentration. The wild-cultivated variety showed a higher concentration of protein (20.73%) compared to the indoor cultivated variety (in the range of 2.62 to 4.69%). Although all three studies used the same Kjedahl's method for protein determination, the protein content greatly differed from each other. The wild *V. volvacea* fruiting bodies in Masitah et al. (2023) especially have the highest protein content of 20.73%. This finding is possibly influenced by the method used by the researcher to obtain the fruiting bodies.

This paper and Zikriyani et al. (2018) cultivated *V. volvacea* in a controlled environment using a specifically designed substrate formulation and quantity, compared to wild *V. volvacea*. Whereas the wild *V. volvacea* was obtained from five different palm oil plantations, and only the fruiting bodies with the heaviest weight were selected for analysis. The natural climate conditions provided by the palm oil plantation have no capacity limit and may have extensively contributed to the growth of *V. volvacea*.

The extraction method and protein determination techniques used in this study could account for the notable differences in protein content observed. Different studies often employ varying protocols, which can lead to discrepancies in reported protein levels. In this study, the Kjeldahl method was used for protein determination, which measures the total nitrogen content and estimates protein content using a conversion factor. However, it is essential to consider that other studies might use different methods,

such as the Dumas method or specific protein assays, which can yield different results. For instance, the Dumas method is a combustion technique that can sometimes provide higher protein values due to its direct measurement of nitrogen (Wang et al., 2022).

Additionally, the type of substrate used for cultivation can significantly influence the nutritional composition of the mushrooms. Substrates such as POEFB fiber pellet, paddy straw, cotton waste, and banana leaves have different nutrient profiles, which can affect *V. volvacea*'s growth and nutrient absorption. Comparing our findings with previous research, it was noted that *V. volvacea* cultivated using wild methods often shows a higher protein content. This might be due to the natural variability and richness of the wild substrates compared to controlled indoor conditions. According to Mortada et al. (2020), mushroom cultivation had a significant impact on the physicochemical composition of the mushroom substrate. This supports the fact that different types of cultivation and substrates, as well as the preparation method of the substrates, might have a different impact on the nutritional composition of the produce. Further research into the specific nutrient composition of each substrate and how they interact with *V. volvacea*'s metabolic processes would provide more insight into these variations.

Researchers also discovered that the various substrate types used produced a variety of nutritional components. The carbohydrate content of indoor-cultivated mushrooms using POEFB fiber was high compared to wild-cultivated mushrooms with raw POEFB. Clearly, cultivating mushrooms with POEFB (fiber or raw bunches) yielded more nutritious value than other substrates such as paddy straw, cotton waste, and banana leaves. This finding aligns well with the findings of Hoa et al. (2015), who showed that a distinct substrate formula significantly influenced the nutritional composition, total colonization, period, fruiting body characteristics, yield, biological efficiency (BE), and mineral contents. Thus, it is strongly suggested that POEFB is a good source of substrate for *V. volvacea* cultivation.

The indoor cultivated variety exhibited a higher moisture content distribution, ranging from 86.0 to 91.49%, whereas the wild cultivated mushroom displayed a moisture content of 71.47%. According to Ganogpichayagrai & Suksaard (2020), moisture content is the amount of loss caused by the drying of water and volatile substances. Therefore, the characteristics of the food product, including its shape, color, texture, taste, and weight, can be associated with its moisture content. It can be proposed that *V. volvacea* can be considered a perishable commodity because of its high moisture content, especially for indoor-cultivated varieties. Fang et al. (2019) supported this finding by describing the high moisture content in the fruiting bodies of *V. volvacea*, which may lead to autolysis, water loss, browning, physical damage, and microbiotic invasion, resulting in its highly perishable characteristics during storage, transportation, and shelf life.

The wild-cultivated mushroom has a higher ash content compared to the indoor-cultivated variety. This indicates that wild-cultivated varieties contain a higher amount of inorganic residue (minerals) compared to indoor-cultivated varieties. Ash refers to the inorganic (mineral) residue remaining after the combustion or complete acid-facilitated oxidation of organic matter in food (Harris & Marshall, 2017). This element indicates that the mushroom contains some nutritionally important minerals, such as calcium, phosphorus, sodium, potassium, and magnesium. According to Harris & Marshal (2017), food ash content can range from 0 to 12%, but fresh foods rarely exceed 5%. As a result, the ash content in *V. volvacea* was typically an acceptable amount, regardless of the cultivation methods or substrate sources used.

Conclusion

The nutrient composition of *V. volvacea* was found to be different depending on the types of cultivation methods used and the chosen substrate material for mushroom growth. It can be concluded that many factors may be involved in the difference in nutritional composition of mushrooms cultivated following different cultivations and substrate utilization. However, in both cultivation varieties, the POEFB proved to be the most effective substrate source for *V. volvacea*, outperforming other substrate materials. It was also revealed that the composition of substrates and the growth conditions also significantly

affected the nutritional composition produced by this mushroom. Besides, the differences in protein content are likely due to both the methods of extraction and determination used as well as the inherent nutritional differences in the substrates. Future studies should aim to standardize methods and explore the detailed nutrient profiles of various substrates to fully understand their impact on the nutritional composition of *V. volvacea*. It is also recommended that more studies involving minimizing the perishable effects of this mushroom be done. Enhancing this mushroom's potential for market commercialization is crucial.

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Author Contribution

NF Amir contributed to the labwork task, first draft and conceptualization of the article. A Mohd-Aris contributed to the supervision, review and editing final manuscript.

Conflict of Interest

Authors declare no conflict of interest in this project.

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