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# MYCOCHEMICAL COMPOSITION OF STRAW PADDY MUSHROOM (*Volvariella volvacea*) GROWN ON USED PALM OIL BUNCH

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**Abstract:** The study objective was to determine the mycochemical composition of paddy straw mushroom (*Volvariella volvacea*) grown on used palm oil bunch. Samples were collected at three growth stages; i.e. button stage, egg stage and elongation stage. Results showed that button stage contained higher protein (27.67%), phenolic content (5.18 mg/100g FW) and antioxidant activity compared to elongation stage, which at 26.33%, 4.58 mg TE/100g FW and 0.80 mg TE/100g FW, respectively. Button stage also contained higher mineral contents, the magnesium (9.16 ppm), potassium (47.23 ppm), zinc (0.68 ppm), iron (1.59 ppm) and calcium (2.52 ppm) compared to elongation stage with values of 8.78 ppm, 42.38 ppm, 0.62 ppm, 0.77 ppm and 2.45 ppm, respectively. Paddy straw mushroom (*V. volvacea*) contained higher nutritional and antioxidant compared to egg stage and elongation stages. Palm oil bunch can be valorised as potential substrates for high nutrient paddy straw mushroom production by local mushroom growers.

Keywords: Straw paddy, antioxidant, phenolic content, ferric reducing antioxidant power.

## Introduction

Paddy straw mushroom (*Volvariella volvacea*) is a world famous edible mushroom delicacy variety that has high demand due various specialities in diet food source and in medication. *V. volvacea* is a popular devour among mushroom lovers for its distinct flavour, pleasant tastes, higher protein content and shorter cropping duration compared to other cultivated mushrooms (Rajapakse *et al.*, 2011). They also contain certain compounds consists of anti-cancer, anti-viral, anti-hyper and hypotensive. They have the unique ability to lower cholesterol levels in the blood (Thiribhuvanamala *et al.*, 2012). This species grows widely and naturally on dead leaves, dead wood, animal dropping, on trees, waste stumps, water-hyacinth, palm oil bunch, water pericarp wastes, banana leaves, cotton waste, paddy straw, palm oil waste and sugarcane bagasse (Roy *et al.*, 2014). According to Thiribhuvanamala *et al.* (2012), the suitable temperature for straw paddy mushroom cultivation is at 30°C to 35°C with 75% to 85% humidity. Usually, its growth from spawning to first harvest of crop may take around 9 to 10 days i.e. button stage (Figure 1a). The first flush constitutes about 70% to 90% of the expected mushroom yield. At the egg stage, the veil starts to break. Small spears, pileus stipe and other tissues are clearly visible in the cross-section (Figure 1b). The button stage is formed within 24-48 hours after egg stage mushroom has been harvested (Figure 1c and 1d). Elongation stage mushroom is not always consumed as food, they are sometimes used as tonic mixtures (Figure 1e and 1f) (Rajapakse, 2011; Eguchi *et al.*, 2015).

Protein in mushroom comprises of essential amino acid; the lysine and leucine (Azevedo *et al.*, 2015). In addition, it contains very low amount of fat, but with large amount of polyunsaturated fatty acid (72% - 85%), which are mainly the linoleic acid, whereby mushroom is considered as healthy food (Mshandete & Cuff, 2007). Fatty acids in residual palm oil used as substrate for growing of the mushroom could stimulate the growth of mycelial and enhance mushroom production (Ozcan *et al.*, 2013). Chemical

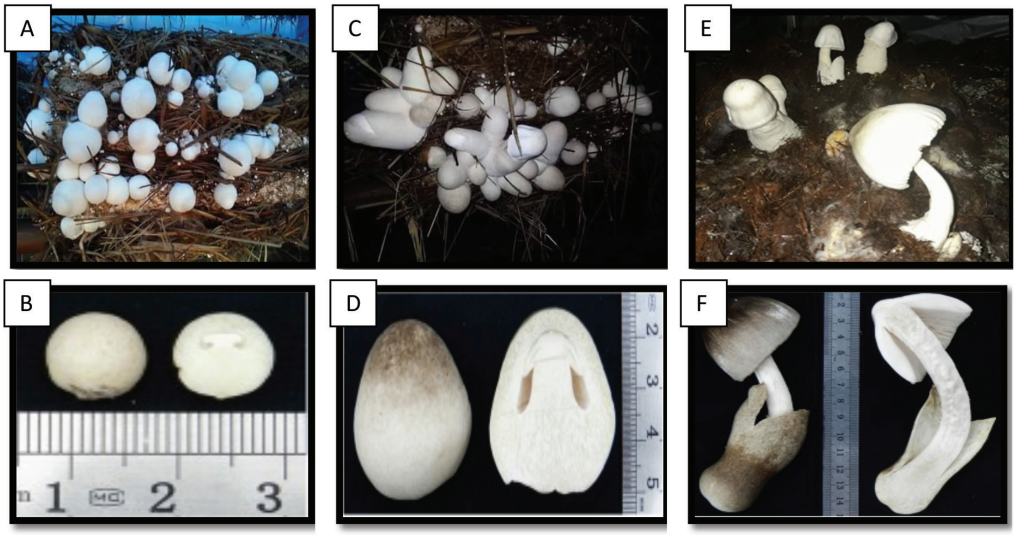


Figure 1: Images showing different growth stages along with cross section of straw paddy mushroom (*Volvariella volvacea*) at (a, b) button stage, (c, d) egg stage and (e, f) elongation stage.

composition continuously changes at different growth stages and post-harvested (Eguchi *et al.*, 2015). According to Kalmis *et al.* (2011), the moisture and nutrient content decreased during growth in mycelium, young fruiting body and in mature fruiting body of mushroom *Tricholonma anatolicum*. Hence being the motivational background, this study objective is to determine the mycochemical properties of *V. volvacea* at three growth stages, namely the button stage, egg stage and elongation stage.

## Materials and Methods

### *Mushroom Harvesting*

The fresh paddy straw mushroom, to be cultivated on used palm oil bunch was obtained from mushroom farm site at Pejabat Pertanian Daerah Padang Terap, Kuala Nerang, Kedah, Malaysia. The legs of fresh mushrooms were carefully dug out using sterilised scalpel. They were then separated accordingly by different growth body stage, which are; button stage, egg stage and elongation stage, harvested on day 10, 12 and 15, respectively. Then divided mushrooms were placed in sterilised sampling bag and were kept at low temperature in ice water boxes to ensure the freshness and to reduce their continuous growth.

### *Mushroom Cleaning*

The mushroom was brought back to laboratory and cleaned according to methods suggested by Thiribhuvanamala (*et al.*, 2012). The fruiting body was cut into half and weighted (FW) before it was stored in freeze drier with a temperature of 20°C. The mushroom was placed in a cool and dry condition in order to avoid microbial growth. After it was stored in the freeze drier for a week, the dried mushrooms was weighed (DW) and then grounded with food processor and the powder form of the sample was again weighed (PW).

- water loss from fresh mushroom = DW - FW
- water loss from dried mushroom = PW - DW

FW is weight of fresh mushroom

DW is weight of dried mushroom after placed in freeze drier

PW is weight of powder form sample

### *Moisture Content Analysis*

Moisture content in mushroom differs in every growth stage of mushroom (Rai *et al.,* 2008). Three grams of mushrooms was grounded and analysed using the MB45 Halogen Moisture Analyser. The moisture content in

food also influences its stability and quality. MB45 Halogen Moisture Analyser being the instrument that was used to determine the moisture content in straw paddy mushrooms. It was determined by thermogravimetric method based on the halogen radiator technology. Moisture content was expressed in percentage by total dry product (dry basis).

### *Crude Protein Content*

The solution in the digestion flask was prepared into alkaline by adding sodium hydroxide, which converts the ammonium sulphate into ammonia gas. The amount of crude protein in paddy straw mushroom was determined using the Kjeldahl method. One gram of grounded dried mushroom was digested by heat with 30 mL of sulphuric acid and catalyst (5 g of potassium sulphate + 5 mg selenium) mixture; this is to reduce the organic nitrogen in the sample to ammonia, which was retained in the solution as ammonium sulphate. Boric acid was added in receiving flask to lower the pH level that converted the ammonia gas into the ammonium ion, and simultaneously converted the boric acid into borate ion. Then, the collected distillate produce was later titrating with 0.1M hydrochloric acid, HCl (Lopez, 2013).

### *Fat Content*

The analysis of fat content was conducted using the Soxhlet Extraction technique. Two grams of dried ground mushroom was weighted in the thimble loosely and 150 mL petroleum ether was added into round bottom flask. This process was meant to make fat extract semi continuously with an organic solvent. The extraction process took around 8 hours on an electro thermal extraction unit. Then, petroleum ether was evaporated on boiling water bath and the extractions were transferred into an oven prepared with 105°C heat for 1 hour. It was later set cooled in a desiccator and the extraction from the flask was weighed. The fat content was measured by weight of fat being removed (Barreira, 2014).

### *Carbohydrate Content*

The amount of total carbohydrate in paddy straw mushroom was determined according to Ezeibekwe *et al.,* (2009).

### *Ash Content*

Ash content analysis was used to determine the mineral amount in the mushroom. Five gram of homogenised mushroom was place in a porcelain dish and placed to dry at 105°C for 3 hours. Then, it was charred over a Bunsen burner until it ceased smoking. The sample was placed in muffled furnace at 550°C for 3 hours until greyish ash is formed. Then the dish was cooled in a desiccator and weighed (Ezeibekwe *et al.,* 2009).

### *Crude Fibre Content*

The fibre content was determined using the acid and alkali digestion method. Crude fibre in the sample was extracted by sulphuric acid and sodium hydroxide. In this analysis, 3g of ground mushroom sample was boiled in 200 mL of $H_2SO_4$ concentration for about 30 minutes. The insoluble residue (not a fibre) was filtered and washed away by hot water rapidly to ensue the alkaline on the residue. After it was dried in an oven at 100 ºC for 3-4 hours, it was heated on oxidizing (blue) flame until the smoke ceased to appear out from of the sample. Then, it was placed in a muffle furnace at 550 °C for 4 hours until a grey ash obtained, then it was cooled and weighed (Ul Haq *et. al.,* 2011).

### *Mineral Content*

Mineral constituent was analysed using the inductively coupled plasma mass spectrometry (ICP-MS). Sample undergoes digestion process before it was analysed by using ICP-MS. 0.5 g of ground sample and 10 mL of concentrated nitric acid was mixed together and was left overnight in a fumed hood. The mixture was heated until red fume disappeared and left to cool in the fume hood. 1 mL of $H_2O_2$ was pipetted into the mixture and was heated for a few minutes to homogenise. At the same

time, the standard was prepared by diluting 5 mL single standard ICP for five elements with 20 mL deionised water. The standard was prepared for 1 ppm until 5 ppm. Once those samples, standard and blank (deionized water) were prepared, they were analysed using ICP-MS instrument. The result was recorded in (mg/L) (Liu *et al.,* 2008, Dursun *et al.*, 2005).

### *Antioxidant Activity*

Powdered forms 10 g of sample was extracted in 70:30 mL ethanol: distilled water ratio in a volumetric flask. The mixture was vigorously shake at 200 rpm for 1 hour at 25°C. The residues were then filtered. The supernatant was recovered and transferred into an aluminium-covered bottle to avoid direct exposes to light and then were stored in a chiller (Maurya, 2010). The antioxidant activity was determined using ferric reducing antioxidant power (FRAP) reagent. The FRAP reagent was prepared by mixing acetate buffer, TPTZ and $FeCl_3.6H_2O$ at ratio of 10:1:1. TPTZ solution was prepared by dissolving TPTZ (powder) with hydrochloric acid (HCl) and warmed in a water bath. TPTZ solution needs to be prepared fresh on the day of analysis. Ferric chloride hexahydrate was prepared by diluting with distilled water and was also made fresh. For sample preparation, 100 µl of sample was mixed with 3 mL of FRAP reagent and 3 ml of distilled water ($H_20$). The prepared sample was kept in a dark place and was incubated at 37°C for 8 minutes. Absorbance of the sample was measured in comparison to a blank at a wavelength of 595 nm spectrophotometer. While for standard preparation (Trolox standard), 10 mg of Trolox powder was diluted with 40 mL methanol (MeOH). 10 µL of sample was mixed together with 3 mL of FRAP reagent and 3 mL of distilled water ($H_2O$). It was later kept in dark storage and was incubated at 37°C for 8 minutes. Calibration curve was prepared using standard solutions of ferrous sulphate at concentration of 0.5-2.5 mmol/L (Gan *et al.,* 2013).

### *Total Phenolic Content (TPC)*

Total phenolic content in the sample was determined using the Folin-Ciocalteu method. For assay, 200 µL samples were mixed with 800 µL $Na_2CO_3$ (prepared by dissolving 7.5 g/l of $Na_2CO_3$ in distilled water) and 1.0 mL Folin-Ciocalteu reagent. After 30 minutes, the absorbance was measured. Standard solution was prepared using 0.01 g gallic acid in 10 mL $H_2O$. Gallic acid, 800 µL of $Na_2CO_3$ and 1 ml of Folin-Ciocalteu reagent was mixed in test tubes. The standard used was gallic acid and was prepared in 12 ppm, 14 ppm, 16 ppm, 18 ppm and 20 ppm. After 30 minutes, the absorbance was being measured. The results of the mean of the three readings were expressed as mg of gallic acid equivalent (Maurya, 2010).

### *Statistical Analysis*

The results obtained were reported as mean + SD of triplicate measurements. Significant differences of multiple comparisons were determined by one-way analysis of variance (ANOVA) followed by Duncan test with $\alpha = 0.05$ using SPSS (Rabita & Fariza, 2013).

## Results and Discussion

### *Moisture Content and Yield Lost*

The percentage of moisture and the yield loss from fresh mushroom to powder form sample at different growth stages are indicated in Table 1. Samples at the button stage was found to contain significantly lowest moisture content of 8.65% ± 0.64 compared to the egg stage (10.30% ± 0.45) and the elongation stage (9.80%±0.21). The high moisture content in the samples, especially at the egg stage, suggests that great care must be taken in their handling as high moisture content promotes susceptibility to microbial growth and enzyme activity. Moisture is simply water diffused in a relatively small quantity and water molecule in samples has strong intermolecular bonding capabilities (Sikorski, 2006). Eguchi *et al.* (2015), revealed that the percentage of moisture of straw paddy mushroom during

Table 1: Percentage of moisture and yield loss in different growth stages in straw paddy mushroom (*Volvariella volvacea*)

| | **Button stage** | **Egg stage** | **Elongation stage** |
|---|---|---|---|
| **Moisture content (%)** | 8.65±0.64[c] | 10.30±0.45[a] | 9.80±0.21[b] |
| **Yield loss (%)** | 89[a] | 90[a] | 93[a] |

*Data explain mean±standard deviation in a column indicated the different letter are significantly different ($p < 0.05$)

button stage was lesser than egg stage (5%), while egg stage was (12%), and the highest was during elongation stage (14%). Water loss from sample during sampling was associated with holding time, temperature and humidity in laboratory environments. Weight changes in freeze dried method were assumed to be due to moisture loss. Besides, weight gains can also occur due to oxidation of unsaturated fatty acids and certain other compounds (Bradley, 2010). Freeze drier was used to reduce the moisture content and water activity in mushrooms in order to reduce the risk of microbial spoilage or deleterious effects on the mushrooms caused by enzyme (Li *et al.*, 2017).

### *Proximate Content*

Results in Table 2 showed that major component in straw paddy mushroom were carbohydrate which ranges from 51.47%±0.00 to 53.14%±0.00, although there were not significant during the three different growth stages. In similar study, Subbiah and Balan (2015) had reported that the percentage of carbohydrate amounting to 57%. Protein content in button stage was found to be significantly higher (27.67% ± 0.58) compared to elongation stage (26.33%±0.58). Previous study by Subbiah and Balan (2015) also recorded that the percentage of protein in edible mushroom to be at 25%. Another study by Eguchi *et al.,* 2015 found that protein content at egg stage was at 32.9g/100 compared to 38.9 g/100 g at elongation stage. Major factor that affect the protein content of mushrooms was level of nitrogen in the substrate (Lopez *et al*, 2013). Button stage contained the highest level of nitrogen and elongation stage recorded the lowest level of nitrogen. Other factors associated with protein content were part of fruit body and harvest location (Eguchi, 2015). Mushrooms are considered as good source of digestible proteins. The protein content of edible mushrooms, besides being species or strain specific, could also vary with the growth substrate (Mshandete & Cuff, 2007). Fibre content in button stage was significantly higher (2.42% ± 0.05) compared to elongation stage (1.39% ± 0.25). According to Thiribhuvanamala *et al.,* (2012), the fibre content in straw paddy mushroom was similar at button and egg stages which is 1.87%, and decreased due to maturity of the mushroom. The changes in the crude fiber content can be associated with the nature of the mushroom fiber that dissociate when they are exposed at certain temperature (Adejumo & Awsanya, 2005). According to Hassan *et al.,* (2007), the breakdown of hemicellulose components, water soluble pectin and hydrocolloids facilitate the changes in crude fiber content.

Ash content in button stage mushroom was significantly lower (6.22 % ± 0.17) compared to elongation stage (6.58% ± 0.13). The ash content attributed in straw paddy mushroom was similar at all growth stages, which is 12.5% (Subbiah and Balan, 2015). Previous study by Teklit (2015) on the nutritional analysis of cultivated mushrooms found that the total ash was 9.41%. Few factors are found leading to change of ash content, which are the duration after harvested, environmental temperature and humidity (Ezeibekwe *et al.,* 2009). Fat content in button stage mushroom was significantly lower (4.32% ± 0.04) compared to elongation stage (5.82% ± 0.01). Subbiah and Balan (2015) also revealed that fat content in straw paddy mushroom was 5.7% and the level of fat

Table 2: Proximate values in different growth stages in straw paddy mushroom (*Volvariella volvacea*)

| | Proximate content (%) | | | | | |
|---|---|---|---|---|---|---|
| | **Moisture** | **Ash** | **Protein** | **Fat** | **Carbohydrate** | **Fibre** |
| **Button stage** | 8.65±0.64[b] | 6.22±0.17[b] | 27.67±0.58[a] | 4.32±0.04[c] | 53.14±0.00[a] | 2.42±0.05[a] |
| **Egg stage** | 10.30±0.45[a] | 6.36±0.17[a,b] | 26.67±0.58[a,b] | 4.49±0.08[b] | 52.18±0.00[a] | 2.54±0.13[a] |
| **Elongation stage** | 9.80±0.21[a] | 6.58±0.13[a] | 26.33±0.58[b] | 5.82±0.01[a] | 51.47±0.00[a] | 1.39±0.25[b] |

*Data explain mean±standard deviation in a column indicated the different letter are significantly different ($p<0.05$)

content decreased as the mushroom matured, which is contrary to our findings.

From soxhlet extraction method and the use of organic solvent in the soxhlet extraction indirectly breaks down certain polyglycerides from the sample into smaller components such as the fatty acids or lipids and fat content. This is credited to a wide variety of lipid compounds (Eguchi *et al.*, 2015). Eguchi *et al.*, 2015 also reported that fat content increased with the maturity of mushroom.

### *Mineral contents*

Due to high level content of most mineral elements within straw paddy mushroom, it attributed as essential for normal metabolic reaction, transmission of nerve impulse, regulation of water and salt balance and rigid bone formation (Ezeibekwe, 2009). The mineral features of this mushroom was categorised as predominant mineral, major mineral and minor mineral. Major mineral found in the similar *Volvariella speciosa* which is grown in Uganda are potassium (K), phosphorus (P), sodium (Na), calcium (Ca) and magnesium (Mg) (Nakalembe *et al,* 2015).

The mineral contents were predominant at button stage compared to other stages (Table 3). Button stage contained significantly higher potassium (K) (47.23 ± 0.39 mg/g) which was a predominant element compared to elongation stage (42.38 ±0.13 mg/g). It was proven by previous study on edible mushroom, where potassium content ranges from 1809.7 mg/100 g to 3354.45 mg/100 g making 38.4–71.4% of RDI (recommended dietary intake) (Nakalembe *et al,* 2015). The higher content might be due to its essential function as a main electrolyte and major cation inside the cell of mushroom (Nakalembe *et al,* 2015). Button stage also contained significantly higher Magnesium (Mg) (9.16 ±0.01 mg/g) compared to elongation stage (8.78 ±0.02 mg/g). Previous study had also found that magnesium is higher followed by calcium (7.14–31.9 mg/100 g) making 1.8–8.0% of RDI (Nakalembe *et al,* 2015). In addition, calcium (Ca) was found to be significantly higher at button stage (2.52 ± 0.02 mg/g) compared to elongation stage (2.45 ± 0.01 mg/g). Similarly, iron (Fe) and zinc (Zn) were significantly higher at button stage i.e. 1.59 ±0.01 mg/g and 0.68 ± 0.01 mg/g compared to elongation stage i.e. 0.77 ±0.01 mg/g and 0.62 ±0.01 mg/g, respectively. Compared to reported data from the previous study, the different of mineral content was influenced by various factors, for instance is strain types, part of mushroom body used, the composition of growth substrate and the factor of environment ambience such as humidity and temperature (Nakalembe *et al.*, 2015).

### *Phenolics and antioxidant activity*

Interestingly, total phenolic compounds were significantly higher at button stage, 5.18 ± 1.83 mg/100g fresh weight (FW), compared to elongation stage (0.51 ± 2.44 mg/100g FW) as shown in Table 4. Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals (Sahu & Saxena, 2013). The main phenolic

Table 3**:** Mineral content in different growth stages in straw paddy mushroom (*Volvariella volvacea*)

| | Mineral content (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | Magnesium | Potassium | Zinc | Iron | Sodium | Calcium |
| **Button stage** | 9.16±0.01[a] | 47.23±0.39[a] | 0.68±0.00[a] | 1.59±0.01[a] | 4.91±0.01[b] | 2.52±0.02[a] |
| **Egg stage** | 8.15±0.03[c] | 40.30±0.12[c] | 0.61±0.00[c] | 1.34±0.01[b] | 4.84±0.03[c] | 2.05±0.03[c] |
| **Elongation stage** | 8.78±0.02[b] | 42.38±0.13[b] | 0.62±0.00[b] | 0.77±0.00[c] | 6.06±0.03[a] | 2.45±0.01[b] |

*Data explain mean±standard deviation in a column indicated the different letter are significantly different ($p < 0.05$)

compounds found in edible mushrooms are phenolic acids, which were divided into two major groups; hydroxybenzoic acids, and hydroxycinnamic acids (Kozarski *et al.*, 2015). The antioxidant capacity and total phenolic content of edible mushroom in young and mature stage have been evaluated with minor differences (Sharma *et al.,* 2015). Previous study from Sudha *et al,* 2008, total phenolic contents in straw paddy mushroom were in the range of 10.05 mg/g dried weight (DW) to 16.72 mg/g dried weight (DW).

Antioxidant was significantly higher in button stage (4.58 ± 0.39 mg/100g fresh weight (FW) as compared to elongation stage (0.80±0.04 mg/100g of FW). Previous study done by Jayakumar *et al.,* (2009) reported that *V. volvacea* species showed appreciable reducing power activities at certain levels of concentration (2-10 mg/mL). Furthermore, Jayakumar *et al.,* 2009 also stated that younger stage of *V. volvacea* species had a greatest ability to reduce the ferricyanide complex to ferrous form. Straw paddy mushroom was one of edible mushrooms that contain high antioxidant activity compare to other edible mushroom. High level of antioxidant substances in straw paddy mushroom was due to calcium carbonate activity (Roy *et al.,* 2014). However, it will decrease as storage time increases with high moisture and temperature differences (Sharma *et al.,* 2015). Thus, holding time need to be reduced in order to prevent loss of antioxidant properties from this mushroom.

*V. volvacea* are well recognised as nutritious food that complement to our daily intake for various nutrients. However, the content of nutrients changes as the mushroom body becomes mature and continues to grow. From this study, it was found that straw paddy mushroom at button stage contained higher chemical constituents and nutritional values compared to other development stages i.e. egg stage and elongation stage. At button stage, significantly higher protein was found while the fat content was significantly lower compared to elongation stage. Potassium was predominantly found in button stage mushroom, as well as, magnesium, zinc, iron and calcium compared to other growth stages. Higher total phenolic content and antioxidant activity measured using FRAP assay were recorded in button stage compared to egg stage and elongation stage.

Table 4**:** Total phenolic content (TPC) and Ferric reducing antioxidant potential (FRAP) in different growth stages in straw paddy mushroom (*Volvariella volvacea*)

| | **Button stage** | **Egg stage** | **Elongation stage** |
|---|---|---|---|
| **TPC (mg GAE /100g of FW)** | 5.18±1.83[a] | 0.82±0.21[b] | 0.51±2.44[c] |
| **FRAP (mg TE /100g of FW)** | 4.58±0.39[a] | 1.18±0.09[b] | 0.80±0.04[b] |

*Data explain mean±standard deviation in a column indicated the different letter is significantly different ($p < 0.05$). **FW is stand for fresh weight of sample

## Conclusion

Paddy Straw mushrooms are well recognised as nutritious food that complement to our daily diet intake for their various nutrients significances. The content of nutrients changes as the mushroom body becomes mature and continues to grow. Different substrate also gives different nutritional benefits to the mushroom. From this study, it was found that straw paddy mushroom grown on palm oil bunch contained higher chemical constituents and nutritional values at button stage compared to other development stages i.e. egg stage and elongation stage. At button stage, significantly higher protein was found while the fat content was significantly lower compared to elongation stage. Potassium was predominantly found in button stage mushroom, as well as, magnesium, zinc, iron and calcium compared to other growth stages. Higher total phenolic content and antioxidant activity measured using FRAP assay were recorded in button stage compared to egg stage and elongation stage. This study demonstrated that palm oil bunch that are usually discarded and dumped in the palm oil plantation itself can be used as a potential substrate to grow straw paddy mushroom. Using this waste material is a mean to manage pest problems and also reduce unnecessary dumping in the landfills. Altogether, discarded palm oil bunch are available by mushroom growers for a very cheap price and thus reduces the operational cost of growing mushrooms.

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