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MYCOCHEMICALS AND PROXIMATE COMPOSITION OF TWO DIFFERENT STAGES IN FRUITING BODY DEVELOPMENT OF *VOLVARIELLA VOLVACEAE* GROWING NATURALLY ON OIL PALM EMPTY FRUIT BUNCHES

MASITAH[1], KRISHNA PURNAWAN CANDRA[2], MUHAMMAD AMIR MASRUHIM[3] AND PINTAKA KUSUMANINGTYAS[4]*

[1]Biology Education Study Program, Faculty of Teacher Training and Education, Universitas Mulawarman, Jl. Muara Pahu, 75119 Samarinda, Indonesia. [2]Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Mulawarman University, Jl. Paser Balengkong, 75119 Samarinda, Indonesia. [3]Chemistry Education Study Program, Faculty of Teacher Training and Education, Universitas Mulawarman, Jl. Muara Pahu, 75119 Samarinda, Indonesia. [4]Chemistry Education Study Program, Faculty of Teacher Training and Education, Universitas Mulawarman, Jl. Muara Pahu, 75119 Samarinda, Indonesia.

**Corresponding author: pintaka@fkip.unmul.ac.id*
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Abstract: This study evaluated the mycochemicals and proximate composition of the mushroom fruiting body of *Volvariella volvaceae* growing naturally on Oil Palm Empty Fruit Bunches (OPEFB) between the egg and mature stages. Phytochemical screening and FTIR were applied for qualitative mycochemicals analysis, meanwhile, quantitative mycochemicals analysis was determined by calculating Total Phenolic Compounds (TPC) and Total Flavonoid Content (TFC). The antioxidant activity of the mushroom was determined by using DPPH radical-scavenging activity. The proximate analysis was done using standard laboratory methods to determine the nutritional content of the mushroom. Mycochemical analysis of mushroom extracts both at the egg and mature stages exhibited the presence of alkaloids, flavonoids, phenolic, terpenoids, and saponin. The TPC and TFC in the mushroom tend to decrease during the growth from egg to mature stage, as well as its antioxidant activity. Proximate analysis revealed that the egg stage of mushrooms contained a higher content of moisture and carbohydrate than those at the mature stage. Meanwhile, the mature stage of mushrooms contained a higher content of protein, ash, and fat. Results from this study show the potential of *V. volvaceae* fruiting body at both the egg and mature stages as sources of natural antioxidants, flavour enhancers and food supplements.

Keywords: FTIR spectra, phytochemical analysis, antioxidant activity, proximate analysis, *Volvariella volvacea.*
Abbreviations: OPEFB = Oil Palm Empty Fruit Bunch.

Introduction

Edible mushrooms are considered a source of essential nutrients and various aromatic flavours. They contain high protein, carbohydrates, vitamins, and minerals, but are low in calories and fat (Nasir & Siva, 2019; Wang & Zhao, 2023). Mushroom proteins contain the 8 essential amino acids (arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, and valine) required by humans (Yuwa-Amornpitak *et al*., 2020). Various edible mushroom species also contain a large number of healthful active ingredients, such as functional polysaccharides, prebiotics, terpenoids, phenolic compounds, and adenosine (Wang & Zhao, 2023). These active ingredients in mushrooms possess several health benefits, such as antioxidant, anticancer, antitumor, immunomodulatory, antimicrobial, anti-coagulation, anti-inflammatory, anti-hypertension, and antidiabetic (Eguchi *et al*., 2015; Kozarski *et al*., 2015). In developing countries, the use of mushrooms for therapeutic purposes is being promoted as a benefit for human health and natural dietary supplements.

The local people, by the palm oil plantation, are taking advantage of collecting the *Volvariella volvacea* grown on the decomposed OPEFB

(Nasir & Siva, 2019; Triyono *et al.*, 2019; Masitah *et al*., 2023). The *V. volvacea*, commonly known as straw mushroom or Chinese mushroom, is the most popular edible mushroom that is widely cultivated in East and Southeast Asia (Elawati *et al.,* 2022). It is consumed due to its delicious taste, pleasant flavour and medicinal value. The nutritional composition of *V. volvaceae* depends on the growth media. The *V. volvaceae* grown on a corncob and rice bran media contains 2.70% crude protein, 8.00% carbohydrate, 1.99% fat, and 2.17% ash content (Elawati *et al*., 2022), while the mushroom grown on used oil palm empty fruit bunches contains 26.67% protein, 52.18% carbohydrate, 4.49% fat, and 6.36% ash (Nasir & Siva, 2019).

The developmental stages of *V. volvaceae* include the formation of a pinhead, tiny button, button, egg, elongation, and maturity (Tao *et al*., 2014). Mushrooms at the egg stages (undeveloped caps) are preferred for consumption because of their sweet, savoury taste, and soft texture when eaten. Meanwhile, the mushrooms at the mature stage are not desirable. Although *V. volvaceae* at the elongation and maturity stages are less desirable, this mushroom has considerable biomass potential. Based on field observations, we collected the *V. volvaceae* mushrooms at the button, egg, elongation, and mature stages from five OPEFB piles at an oil palm plantation, and we obtained the highest fruit body weight of mushroom is at the mature stage (Table S1 in supplementary material).

The phytochemicals and proximate composition of *V. volvaceae* at the different fruiting body development stages have been reported (Eguchi *et al*., 2015; Nasir & Siva, 2019). However, a shortage of information on differences in the mycochemical and proximate composition of *V. volvaceae* fruiting bodies between the undeveloped caps (button and egg stages) and developed caps (mature stages), especially the mushroom growing on decomposed OPEFB. This study aimed to evaluate the mycochemicals and proximate composition of the mushroom fruiting body growing naturally on OPEFB between the egg and mature stages. The mushroom mycochemicals with antioxidant activity were determined for human health. The information obtained contributes to utilizing *V. volvaceae* in both egg and mature stages, which have considerable potential for functional foods.

Materials and Methods

Mushroom Materials and Sample Preparation

Fruiting bodies samples of wild *V. volvaceae* at the egg stage and maturity stage were collected from decayed oil palm empty fruit bunches in an oil palm plantation area, in East Kalimantan region, Indonesia. The morphological features of *V. volvaceae* are presented in Figure 1. The mushrooms were cleaned of soil with a soft brush without washing. Before nutritional and mycochemical analysis, all samples were freeze-dried and ground into powder.

Phytochemical Screening

The mushroom extraction was done by maceration of mushroom powder with methanol, ethyl acetate, and n-hexane as solvents at a ratio of sample: Solvent was 1:10. The sample was then shaken using a shaker for 48 hours at room temperature. Each successive extraction was filtered using Whatman filter paper to separate the marc from the extract and the solvent was recovered with a rotary evaporator. Finally,

(a) (b)

Figure 1: Image showing different growth stages of *Volvariella volvaceae* at (a) egg stage and (b) mature stage

a phytochemical test to detect the presence of flavonoids, alkaloids, terpenoids, saponins, steroids, and phenolics was applied using standard qualitative procedures (Trease & Evans, 2009).

Total Phenolic (TPC) and Total Flavonoid Compound (TFC) Assay

Only the methanolic extract of the mushroom was assayed for TPC and TFC by spectrophotometry. One mL of methanolic extract (20 mg/mL) was added with one mL of Folin-Ciocalteu reagent (50% v/v), and mixed thoroughly for 3 mins; then, two mL saturated Na_2CO_3 was added. The mixture was allowed to stand for 30 mins at room temperature. The absorbance of each mixture was measured at 750 nm (GENESYS 10S UV-Vis spectrophotometer, Thermo Scientific, USA). The concentration of Total Phenolic Compounds was measured by plotting the calibration curve of a gallic acid standard, determined as milligrams of gallic acid equivalents (GAE) per gram of dried mushroom (mg GAE/ g dw).

The TFC of the methanolic mushroom extract was measured using quercetin as standard. One mL of the extract (20 mg/mL) was added with four mL of distilled water and 0.3 mL of 5% $NaNO_3$. The mixture was allowed to stand for five min at room temperature. Then, 0.3 mL of 10% $AlCl_3$ was added and mixed well. The solution was allowed to stand again for 6 minutes. The mixture was then added with 2 mL of 1 M NaOH and 2 mL of distilled water. The absorbance was measured at 510 nm (GENESYS 10S UV-Vis spectrophotometer, Thermo Scientific, USA). The TFC was determined as milligrams of quercetin equivalents (QE) per gram of dried mushroom (mg QE/g dw).

Antioxidant Activity assay

The free radical-scavenging activities of mushroom extract were conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. Briefly, 1 mL of each mushroom extract with concentrations of 20 mg/mL was mixed with one mL of DPPH (0.004%) solution in methanol. The mixture was shaken vigorously and left to stand for 30 mins in the dark at room temperature and the absorbance was then measured at 517 nm against a blank using a UV-visible spectrophotometer (GENESYS 10S UV-Vis spectrophotometer, Thermo Scientific, USA). The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

where A_{blank} and A_{sample} are the absorbances of the control reaction (containing all reagents except the test extract) and the absorbance of the test extract, respectively.

Proximate Composition Analysis

The proximate composition of the mushrooms was analysed for nutritional composition (moisture, proteins, fat, carbohydrate and ash) using the AOAC procedures (AOAC, 2005). The moisture content was determined by drying in a hot air oven at 100 ± 5°C to a constant weight. The crude protein content was determined by the Kjeldahl method using a conversion factor of 4.38 instead of the common factor of 6.25, as mushrooms contain significant non-protein nitrogen. The fat content was determined by the Soxhlet method using n-hexane. Finally, the ash content was determined by incineration at 550°C, and total carbohydrates were calculated by difference.

Fourier Transform Infra-Red (FT-IR) Analysis

The freeze-dried samples were grounded into a fine powder, and mixed uniformly with KBr, and pressed into a tablet using a tablet press. The IR spectra were run and taken from the FT-IR Spectrometer (Perkin Elmer, Waltham, USA) with a thermal detector. The samples were scanned between the wavelength of 400 and 4000 cm^{-1} with a resolution of 4 cm^{-1}. The spectra were further imported into OriginPro 8.5.1 (Origin Lab, Northampton, MA, USA) and subjected to (0,1) normalisation.

Statistical Analysis

All samples were analysed in triplicate and expressed as means ± SD. All data were analysed using a two-tailed independent t-test ($p < 0.05$).

Results and Discussions

The Mycochemicals Characteristics of V. volvacea Fruiting Bodies

Qualitative mycochemicals screening of the *V. volvacea* growing naturally on OPEFB at different growth stages is shown in Table 1. The results showed that the extract of the mushroom *V. volvaceae* possessed a variety of secondary metabolites, including phenolic compounds, alkaloids, flavonoids, saponin, and terpenoids. The active substances that are inside the mushrooms depend on the growth stages (Eguchi *et al.*, 2015), and the type of bioactive compounds found in each solvent depends on the solubility of the compounds in the solvent. Alkaloids were present in all organic solvents used in this study for both egg and mature stages because several types of alkaloid compounds can dissolve in polar and non-polar solvents. Alkaloids commonly found in methanol extract are quaternary alkaloids (Golkiewicz & Gadzikowska, 1999). Unger *et al.* (1981) found quaternary alkaloids in mushrooms identified as muscarine and choline. Several alkaloids containing phenolic hydroxyl or carboxyl groups which had polar properties tend to be extracted in semi-polar solvents, including methanol and ethyl acetate (Yubin *et al.,* 2014), and the free alkaloidal base tends to be found in a non-polar solvent, such as n-hexane. The presence of alkaloids in the mushroom fruiting body has antimicrobial effects by inhibiting DNA topoisomerase (Sulaiman *et al.*, 2022).

In this study, flavonoids were only detected in methanol extracts for both egg and mature stages. Mushrooms are rich in flavonoids and other simple phenolic compounds which exhibit a wide range of biological effects as antioxidant, anti-inflammatory, and anti-proliferative activities (Wu *et al.*, 2016). Furthermore, phenolic was confirmed present in methanol and ethyl acetate

Table 1: Characteristics of mycochemicals of *V. volvaceae* fruiting bodies at the egg and mature stages growing naturally on OPEFB

Mycochemicals Characteristics	Methanol Extract		Ethyl Acetate Extract		n-Hexane Extract	
	Egg	Mature	Egg	Mature	Egg	Mature
Qualitative						
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	-	-	-	-
Phenols	+	+	+	+	-	-
Saponins	-	-	-	-	+	+
Steroids	-	-	-	-	-	-
Terpenoids	-	-	+	-	-	-
*Quantitative**						
Total Phenolic Compound (mg GAE/g dw)#	7.14 ± 0.97	2.15 ± 0.02				
Total Flavonoid Compound (mg QE/g dw)#	13.79 ± 2.19	6.65 ± 0.72				

Notes: *Data (means ± SD) were calculated from three replications. The egg and mature stage data at each row were significantly different (t-test, $p < 0.05$).

extracts. The phenolic compounds extracted in methanol commonly have low molecular weights and medium polarity levels. Phenol is the main compound that is thought to be related to the antioxidant effects of mushrooms. Antioxidant compounds prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis. Antioxidant properties found in many mushrooms are generally in the form of phenolic acid (Kozarski *et al.*, 2015). These phenolic acid compounds are typically a component of a complex structure like lignins and cellulose-containing in OPEFB. Accumulation of phenolic compounds in mushroom fruit bodies was likely due to the mushroom absorbing the phenolic acid compounds from OPEFB during its fruiting body development (Yahaya *et al.*, 2017; Satriono *et al*., 2022).

Phenolic compounds and alkaloids are also detected in ethyl acetate extracts. Ethyl acetate is a semi-polar solvent which is also capable of extracting terpenoids. In this study, terpenoid compounds were only detected in ethyl acetate extract at the egg stage of *V. volvaceae* fruiting body. In basidiomycetes, including *V. volvaceae*, the biosynthetic pathway of terpenoids is primarily synthesised through the mevalonate pathway which is initiated from acetyl Co-A (Wang *et al.*, 2021). The amount of acetyl coenzyme A (acetyl-CoA) is a signature of growth and promotes its utilisation for lipid synthesis, including terpenoids (Shi & Tu, 2015). Under survival states (mature stage), acetyl-CoA is preferentially directed to promote mitochondrial-dependent activities such as the synthesis of ATP. Consequently, terpenoids are not synthesised in this mature stage. In this study, saponin was only detected in n-hexane extracts, but steroid was not detected in all extracts. The presence of saponin in n-hexane extract indicated that saponin compounds contained in this mushroom have a hydrophobic group, such as aglycon (sapogenin). Saponins were present in all mushrooms (Hossen *et al.,* 2022). In our study, saponins were detected in both the egg and mature stages. The presence of these compounds stimulates the fruiting bodies of edible mushrooms (Magae, 1999).

The presence of alkaloids, flavonoids, triterpenoids, saponins and phenolic compounds indicated potential antioxidant activity in *V. volvaceae* fruiting bodies. These secondary metabolites can act as free radical scavengers through various mechanisms. Nurhasnawati *et al*. (2019) found that there is a positive correlation between total phenolic content and flavonoid with antioxidant activity. In this study, we compared the contents of Total Phenolic Compounds (TPC) and Total Flavonoid Content (TFC) in the methanolic mushroom extracts between the egg and mature stages with the results as shown in Table 1. The results indicated that the TPC and TFC in *V. volvaceae* were higher at the egg stages compared to the mature stages ($p < 0.05$). This exhibited that the content of TPC and TFC varied depending on the developmental stages of the mushroom fruiting body. Our results were similar to those reported by Nasir & Siva (2019) that *V. volvaceae* at the button stage showed higher phenolic content compared to the elongation stage.

Many publications reported the correlation between the content of TPC and TFC with antioxidant activity (Boonsong *et al.*, 2016; Dulay *et al.*, 2016; Nurhasnawati *et al.,* 2019). In this study, the antioxidant potential of *V. volvaceae* extracts is measured by using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity. The results of the DPPH radical-scavenging activity of mushroom extracts between egg and mature stages were depicted as the percentage as shown in Figure 2. The results indicated that *V. volvaceae* at the egg stages presented the stronger DPPH radical-scavenging activity (57.75 ± 6.93%) at 20 mg/mL compared to the mature stages (32.95 ± 0.97%). These results, however, showed that the *V. volvaceae,* both at the egg and mature stages, could serve as a food rich in natural antioxidants and can be utilized as a functional food for human health benefits.

Proximate Composition

The proximate compositions of *V. volvaceae* growing naturally on OPEFB at the egg and mature stages are shown in Table 2. The content of moisture and carbohydrate decrease during the growth stages from egg to mature stage, but the content of protein, ash, and fat increase significantly ($p < 0.05$). Samples at the egg stage were found to contain higher moisture content of 71.47 ± 1.22% compared to the mature stage of 61.04 ± 1.45%. The moisture content in this work was less than the moisture content reported by Elawati *et al*. (2022) which is 85.14%. This may be caused by climate conditions during mushroom collection, which was in the dry season with low humidity (73–85%) and higher temperature ambient (33–35^{o}C).

The higher moisture content in mushrooms at the egg stage was related to the higher content of carbohydrates (8.78 ± 0.17%) compared to the content of carbohydrates at the mature stage (4.53 ± 0.11%). The higher carbohydrate content at the egg stage causes a sweeter taste and makes the mushrooms at this stage desirable for consumption. This can be explained that most edible mushrooms contain mannitol which is thought to function as an osmoregulator in mushrooms (Zhao *et al.*, 2019). Mannitol, commonly called "the mushroom sugar", is the main carbohydrate storage in the mushroom that encourages the influx of water from the environment to support turgor pressure during mushroom fruiting bodies developments

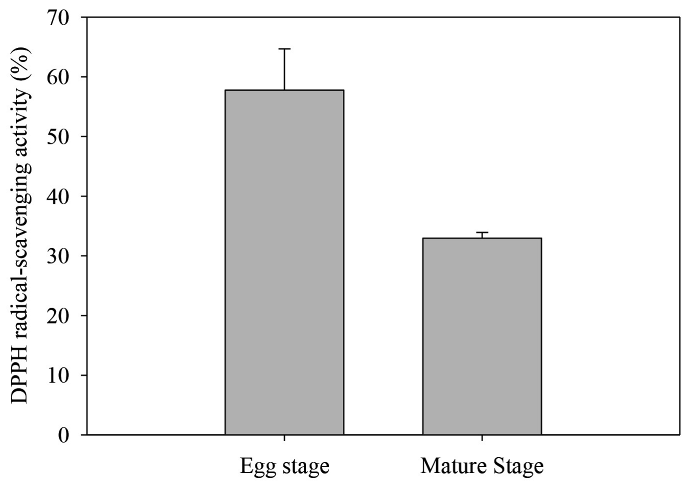

Figure 2: DPPH radical-scavenging activity of dried *V. volvaceae* extract

Table 2: Chemical composition (% db) of two stages of *V. volvaceae* fruiting body mushroom growing on OPEFB

Proximate Composition*	**Egg Stage**	**Mature Stage**
Moisture	71.47 ± 1.22	61.04 ± 1.45
Carbohydrate	8.78 ± 0.17	4.53 ± 0.11
Ash	6.57 ± 0.01	11.66 ± 0.05
Protein	20.73 ± 1.16	27.52 ± 1.79
Fat	2.43 ± 0.14	3.56 ± 0.14

Notes: Data (means ± SD) were calculated from three replications. *The egg and mature stage data for each chemical composition were significantly different (t-test, $p < 0.05$).

(Zhou *et al*., 2016), so it can retain water in tissue. As reported by Kitamoto *et al.* (1999), mannitol content increased until the middle stage of development and then decreased with maturation. This is according to our finding that reduced carbohydrate content in mushrooms followed by reduced water content at the mature stage indicates reduced water-holding compounds in mushrooms.

The content of carbohydrate found in the current results were much lower than those in the same species of mushroom cultivated on used palm oil bunch, as reported by Nasir and Siva (2019), which was 52%. This is because *V. volvaceae* used in this study was a wild mushroom taken from oil palm empty fruit bunches decomposed naturally in oil palm plantations without adding any nutrients for its growth. Srikram and Supapvanich (2016) found that edible cultivated mushrooms possess higher nutritional value than those edible wild mushrooms. However, our results were similar to *V. volvaceae* cultivated on a corncob and rice bran media as reported by Elawati *et al.* (2022) is 8%, but it was lower with all wild edible mushrooms from Northeast Thailand as reported by Srikram and Supapvanich (2016) in ranging from 9.56% to 27.67%. These differences might have been dependent on climate differences, wild or cultivated, species of mushroom and geographical location (Srikram & Supapvanich, 2016).

In this study, the content of protein, ash, and fat in *V. volvaceae* was not much different from *V. volvaceae* cultivated on used palm oil bunch as reported by Nasir and Siva (2019), in which the protein content of mushrooms in the egg stage was 26.67%, the ash content was 6.36%, and the fat content was 4.49%. Similar to the report by Eguchi *et al.* (2015) that the content of protein, ash and fat in *V. volvaceae* increased in increasing levels of maturity. Zhang *et al.* (2021) stated that edible fungus protein contains all kinds of amino acids. Mohd Joha *et al.* (2021) found a total of 16 amino acids in both immature and mature stages of *V. volvaceae*, which are important in producing the flavour and taste of the mushroom. Furthermore, the ash content in mushrooms indicated the presence of mineral nutritional elements that are useful for the necessary activators or active agents in many enzyme reactions in *V. volvaceae* (Chen *et al.,* 2019). Hence, these findings showed that *V. volvaceae* at the mature stage is a good source of protein and minerals which possess attractive nutritive and supposed health-promoting properties. Additionally, due to its low content of fat, this mushroom is a good source of dietary supplements for people who has a history of heart disease and obesity.

The compositions of the substrate greatly influenced the mushroom's nutritional content in its growth medium (Mortimer *et al.,* 2021). In this study, *V. volvaceae* decomposed complex organic carbon contained in OPEFB (including lignin, hemicellulose and cellulose) into simpler macromolecules (carbohydrate, protein, fat, and other substances) that will be available for the growth and development of mushrooms. Among those complex organic carbon sources, lignin is the hardest component to be degraded by this mushroom because *V. volvaceae* lacks an efficient ligninolytic enzyme system (Bao *et al.,* 2013). Hence, *V. volvaceae* prefers substrates with high cellulose and low lignin contents and produces a variety of cellulolytic enzymes for cellulose degradation (Suwannarach *et al*., 2022). Rice straw is known as the best substrate for *V. volvaceae* because it contains lower lignin of 14.3% (Luo *et al*., 2019) compared to OPEFB with a lignin content of 24.12% (Triyono *et al.,* 2019). However, *V. volvaceae* can grow well naturally in OPEFB due to high cellulose content (39.15%) which was similar to cellulose content in rice straw (36.4%) (Luo *et al.,* 2019; Triyono *et al.*, 2019). These suggested that the abundant OPEFB waste can be the main carbon source for the sustainable growth of this mushroom species in nature.

The low carbohydrate content in *V. volvaceae* as reported in this study was probably due to the high lignin content in the substrate because the *V. volvaceae* samples used in this study were taken from one-month-old OPEFB piles. The carbohydrate content in *V. volvaceae* grown in OPEFB can be increased by improving the chemical composition of the substrate, particularly by reducing the lignin content in OPEFB. Suwannarach *et al.* (2022) proposed to use a primary decomposer, including bacteria and fungi, which are capable of producing a variety of ligninolytic enzyme systems (lignin peroxidase, manganese peroxidase, and laccase). Many researchers have successfully isolated ligninolytic bacteria and fungi from OPEFB, such as *Paenibacillus* sp. (Tahir *et al.*, 2019), *Metarhizium anisopliae* (Sahid & Kusumaningtyas, 2023), *Lichtheimia ramose* and *Neurospora crassa* (Tahir *et al.,* 2019), etc. These ligninolytic microbes can play a role as primary lignin decomposers of OPEFB before *V. volvaceae* production. Therefore, the cultivation method which can be used to enhance the production of *V. volvaceae* is by using substrate composting. In advance, the mycochemicals content as well as antioxidant activity in *V. volvaceae* also can be increased by adding calcium carbonate as the best additive for *V. volvaceae* growth (Sudha *et al*., 2019)

FTIR Spectra of Egg and Mature Stage of V. volvaceae

FTIR spectroscopy analysis reveals the presence of different functional groups of bioactive and biochemical compounds present in the mushroom fruit bodies at the egg and mature stages. The FTIR spectra profiles of *V. volvacea* at the egg and mature stage are similar unless the transmittance value (Figure 3). These profiles could explain the shifting concentration (increase or decrease) of mycochemicals and proximate composition from the egg to the mature stage as described in Tables 1 and 2. The description of FTIR spectra characteristics in Figure 2 are followed the peak description by Wilson *et al.* (1988) as shown in Table 3

The FTIR analysis showed several band peaks that differ in their relative intensity. The obvious differences were revealed in the spectral regions at 3100–2255 cm^{-1}, 1645–1559 cm^{-1}, and 1163–520 cm^{-1}. The higher band intensity in lipids-related bands (3100–2255 cm^{-1}) was observed in the mushroom fruiting body spectrum at the mature stage which may lead us to conclude that the lipid content is higher. A band at 1559 cm^{-1} in the mushroom sample at the egg stage was shifted to a higher frequency (1582 cm^{-1}) in the mushroom sample at the mature stage which was attributed to the change in the composition of free amino acids. Mushrooms contain considerably high amounts of free amino acids, which give them a unique natural flavour (Mau, 2005). Peak regions at 2954, 2930, 2859, and 1582 cm^{-1} are also characteristic of the *L*-glutamic acid spectrum which is responsible for the umami

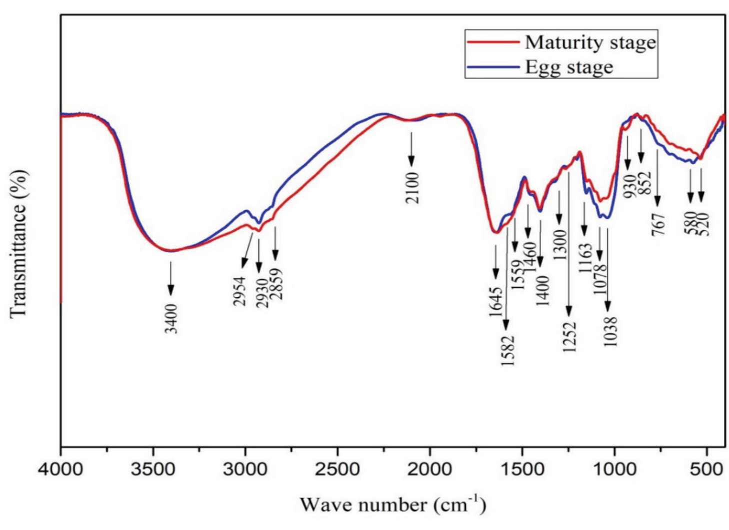

Figure 3: FTIR spectra of *V. volvaceae* fruiting body between the egg and mature stages

Table 3: FTIR bonds' peak for a dried powdered sample of *V. volvaceae* fruiting body (Wilson *et al.*, 1988)

Wavenumber (cm^{-1})	**Band Assignment**
3500–3200	O–H stretching vibration (alcohol, phenol)
3000–2800	H–C–H stretching vibration of lipids
1600–1450	C–C=C stretching of aromatic rings
1640	C=O stretching vibration of amide I (protein)
1544	N–H bending vibration and C–N stretching vibration of amide II (protein)
1500–1440	H–C–H bending
1410	Polysaccharides
1200–900	Polysaccharides
800–400	Polysaccharides

taste in mushrooms. Mau (2005) found that the value of umami taste in *V. volvaceae* fruiting body dramatically increased with the cap opened (mature stage). This indicated that the *V. volvacea* fruiting body at the mature stage can be considered as a raw material for producing natural flavour enhancers. The higher band intensity in polysaccharides-related bands (1163–520 cm^{-1}) observed in the egg stage indicated that the polysaccharides content in this stage is higher. The main polysaccharides present in these mushrooms are β-glucans, which was assigned by a peak around 1163–767 cm^{-1}. The mushroom β-glucans have beneficial effects on human health such as immunomodulatory and anti-inflammatory (Cerletti *et al.*, 2021). Even though the total carbohydrate content in *V. volvaceae* at the mature stage was lower, it is still feasible to be a source of β-glucans due to the larger fruiting body weight. The β-glucans extracted from the mushrooms can be further processed into the form of capsules, tablets, powders or even gummies for food and dietary supplements for the market globally.

Conclusion

The mycochemicals content of *V. volvacea* is shifting to decrease during the growth from the egg stage to the mature stage, as well as two proximate compositions, i.e., moisture and carbohydrate content. On the other hand, other proximate compositions, i.e., protein, fat and ash are increased. The *V. volvaceae* at the egg stage has higher potential as a functional food as it shows higher antioxidant activity due to the higher mycochemicals content. However, *V. volvaceae* at the mature stage still has the potential as a source of natural antioxidants for medicine, cosmetics, and flavour enhancer. The findings showed that the biomass of *V. volvaceae* both at the egg and mature stages growing naturally in OPEFB could be a raw material for the production of functional food, medicine, cosmetics and flavour enhancer due to its mycochemical content and nutritional value. In addition, the utilisation of OPEFB as a substrate for production of *V. volvaceae* biomass could reduce, reuse, and recycle the sustainable management of agro-industrial wastes.

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