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Physical quality, amino acid contents, and health risk assessment of straw mushroom (*Volvariella volvacea*) at different maturity stages

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Abstract

Volvariella volvacea, also known as straw mushroom, is an edible mushroom, and a highly nutritious food source. In Malaysia, tremendous increase in the consumption of this mushroom is due to its characteristics; short cropping duration, distinct flavour, and pleasant taste. During harvesting, the maturity of *V. volvacea* is vital to obtain high quality produce. As the mature stage quickly succeeds the immature stage, farmers usually collect both the immature and mature stages at harvest. Thus, the objective of the present work was to evaluate the physical quality, nutrients, and health risk assessment of *V. volvacea* at both maturity stages. The mushrooms were cultivated on composted empty fruit bunch (EFB), and harvested after a week at immature (button) and mature (veil opening) stages. The absence of spores at the button stage could lower the metabolic activity after harvest, thus decelerating the deterioration rate. Button stage mushrooms showed significantly higher firmness and higher content of glutamic acid. In term of safety, both stages were within the dietary intake limit for heavy metals. In conclusion, the button stage could be suitable and practical for consumption and commercialisation.

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Introduction

Volvariella volvacea, commonly known as straw mushroom, has become one of the most important cultivated mushrooms in the world due to its pleasant taste, distinct flavour, high content of protein, and short cultivation period of approximately two weeks (Rajapakse, 2011; Thiribhuvanamala *et al.,* 2012; Thakur and Singh, 2014). However, its yields are much lower as compared to other mushrooms (Ahlawat *et al.,* 2010). It requires a high temperature range within 30 - 37°C for hyphal development and fructification (Rajapakse, 2011; Roy *et al.,* 2014; Thakur and Singh, 2014).

The developmental stages of *V. volvacea* include the formation of pinhead, tiny button, button, egg, elongation, and maturity (Chang and Miles, 2004). Button and egg are the most preferred by consumers, and often sold at premium price. They are consumed at this stage due to its pleasant flavour and aroma with good taste and texture (Chang and Miles, 2004; Jamjumroon *et al.,* 2012; Ahlawat *et al.,* 2014). However, the dramatic changes from the button stage to opening stage are the main problem during postharvest of *V. volvacea* (Rai and Arumuganathan, 2008). The cap / veil opening would form within just a day from the button stage. Li and Chang (1982) reported that the crude protein content may vary at different maturity stages in *V. volvacea. V. volvacea* could provide all the essential amino acids required for the human diet, especially lysine and leucine, which are usually absent from most staple foods (Chang and Busswell, 1996). Hence, it becomes a crucial part to identify the content of nutrients at different maturity stages of *V. volvacea*.

Recently, mushrooms had become an indicator to determine heavy metal pollution (Tüzen, 2003). They are physiologically able to absorb nutrients from surrounding, including organic matters, which might expose them to heavy metal accumulation (Sithole *et al.,* 2017; Sun *et al.,* 2017). As they grow, they accumulate different amounts of heavy metals at different maturity stages, and should be monitored periodically (Khani *et al.,* 2017). Therefore, the objective of the present work was to evaluate the quality and safety intake limit through health risk assessment of *V. volvacea* at button and veil opening stages.

Materials and methods

Cultivation and harvest

The spawn used for the cultivation of *V. volvacea* was purchased from the Department of Agriculture, Padang Terap, Malaysia. Spores were

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grown in the spawn medium consisting of cotton, paddy straw, and other agricultural wastes that contain cellulose, until they were ready to be used on cultivation bed. The cultivation of *V. volvacea* was conducted at Herbal Garden, University Agricultural Park, Universiti Putra Malaysia. Fresh empty fruit bunches (EFB) of oil palm of about 1 d after fruit bunch removal were composted for 9 d, and watered every 3 d with temperature around 30 - 38°C. The EFB were turned over alternately with an adequate amount of water until the EFB surfaces were evenly moistened during the composting process. The average weight of EFB used was around 3 - 5 kg, with the height of the compost EFB of around 15 cm from the ground. The size of the EFB bed was around 0.8 × 2.1 m. They were incubated with a polysheet plastic for 8 d. Stake of curved pipes were arranged on the EFB bed for mushroom growth. Approximately, a month is needed to completely compost the EFB. The mushrooms could be seen growing on the EFB bed medium within 1 w, and ready for harvest.

In this experiment, the mushrooms were harvested at the button and veil opening stages. Uniform sizes of 3 - 4 cm diameter, and damage-free mushrooms were selected, and dirt/debris on the mushrooms were removed before storage. The mushrooms were handled gently and immediately to avoid major physical damage and deterioration.

Quality and components of Volvariella volvacea at different maturity stages

Firmness

The firmness of the mushrooms was measured following the method described by Zivanovic *et al.* (2003) with slight modification, with an Instron Universal Testing Machine (Model 5543P5995, Instron Corp. Minneapolis, USA). The measurement was conducted by piercing on the upper surface of the button tips, and at the centre of the pileus part during the veil opening stage. The mushrooms were punched with a 6 mm diameter probe, at a 3 mm depth with a crosshead speed of 20 mm/min. The reading was recorded in Newton (N) using Instron Merlin software version M12-13664-EN.

Ultrastructure

The ultrastructure of lamella or gills in both button and veil opening stages were observed under a scanning electron microscope (SEM) using Karnovsky's fixative method described by Zivanovic *et al.* (2000) with slight modification. Samples were cut into slices, and fixed in 2% glutaraldehyde for 2 h at 4°C, followed by washing with 0.1 M sodium cacodylate buffer for three times at 30 min intervals. Thereafter, samples were fixed in 1% osmium tetroxide for 2 h at 4°C. Samples were repeatedly washed with a 0.1 M sodium cacodylate buffer for another three times. Dehydration of samples was conducted using dehydration series of 35, 50, 75, 95, and 100% acetone at room temperature. Lastly, the samples were dried through critical point dried followed by mounting on the stub, and sputter coated with gold. The samples were observed under JSM-IT 100 InTouch Scope™ (JEOL SEM, Japan). The size of basidiospores at the button stage, and mature spores at the veil opening stage were measured using InTouch Scope™ software for three replicates.

Amino acid analysis

The extraction method for both maturity stages of *V. volvacea* followed the AOAC (1990) method with slight modifications. About 0.2 g of mushroom samples were weighed, added with HCl, and heated in an oven for 24 h. Internal standard (Sigma-Aldrich) was added into the samples, and analysis of amino acids was performed by using HPLC (Waters, Alliance 2659) with fluorescence detector. The column used was AccQ tag column (3.9 × 150 mm) with three mobile phases; mobile phase A was Eluent A - AccQ Tag Eluent A:deionised water (1:10), mobile phase B was acetonitrile, and mobile phase C was water. The column temperature was 36 ± 1°C for 10 µL volume of injections. The detection wavelengths were Ex: 250 nm, and Em: 395 nm, at a flow rate of 1 mL/min. The calibration linearity (R^2) was equal to 1. The essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) and non-essential amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, hydroxyproline, serine, and tyrosine) were tabulated from duplicate sample of button and veil opening maturity stages.

Metals and health risk assessment

Heavy metals

Analysis of heavy metals was performed using the wet ashing technique following the method described by Tüzen (2003). One gram of dried mushroom sample was digested with a mixture of 8 mL of nitric acid (HNO_3), 2 mL of sulphuric acid (H_2SO_4), and 2 mL hydrogen peroxide (H_2O_2). The mixture was heated to 150°C for 4 h, and filled with deionised water until the total volume reached 25 mL. The same process was repeated on blank for three replicates. Standards of cadmium (Cd), copper (Cu), iron (Fe), Nickel (Ni), lead (Pb), and zinc (Zn) were prepared in 0, 1, 3, and 5 ppm for each metal, and Fe (0, 5, 10, and 30 ppm). The contents of Cd, Cu, Fe, Ni, Pb, and Zn were analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES). Heavy metal concentration in gram sample was calculated using Eq. 1:

$$\frac{C \times D.F.}{W} \quad \text{(Eq. 1)}$$

where, C = concentration from ICP-OES in (mg/L), D.F. = dilution factor (total volume of sample solution) (mL), and W = sample mass (g).

Estimated daily intake (EDI)

The estimated daily of intake (EDI; mg/kg/day) of *V. volvacea* at both maturity stages for all heavy metals (Cd, Cu, Fe, Ni, Pb, and Zn) was estimated using Eq. 2:

$$EDI = \frac{CM \times WF}{Bw} \quad \text{(Eq. 2)}$$

where, C_M = concentration of heavy metals in mushrooms (µg/g on a fresh weight (FW) basis), W_F = daily average consumption (300 g), and B_W = average body weight of consumer (60 kg).

The oral reference dose (RfDo): Cd = 1.00, Cu = 40.0, Fe = 700, Ni = 20.0, Pb = 4.00, and Zn = 300 based on EPA (2000a; 2015) was used in the present work to evaluate the EDI of heavy metals in *V. volvacea* during immature and mature stages.

Target hazard quotient (THQ)

The THQ is important to measure the health risk from the consumption of this mushroom. People that consume these mushrooms are unlikely to expose from the obvious adverse effects if the ratio of THQ is less than 1, and vice versa. The formula (EPA, 2000b; Aloupi *et al.,* 2012) is described in Eq. 3:

$$THQ = \frac{EFr \ X \ ED \ X \ FI \ X \ MC}{RfDo \ X \ BW \ X \ AT} \ X \ 10^{-3} \quad \text{(Eq. 3)}$$

where, EFr = exposure frequency (365 days/year), ED = exposure duration (70 years), FI = food ingestion rate (300 g/person/d) assumed for adult, MC = metal concentration based on fresh weight basis (mg/kg FW), RfDo = oral reference dose as mentioned above, BW = average body weight (60 kg for adults), and AT = average time for noncarcinogens (365 days/year × ED).

Experimental design and statistical analysis

The experiment was conducted using completely randomised design, and the statistical analysis was conducted using *t*-test in triplicates (means ± SE) using the statistical software SAS 9.4 version.

Results and discussion

Quality and components of Volvariella volvacea
Firmness

The preferences for mushrooms usually depend on both physical and chemical properties, especially firmness (Arumuganathan *et al.,* 2010). The button stage of *V. volvacea* showed significantly higher firmness (77.96%) as compared to the veil opening stage (Figure 1). According to Zivanovic *et al.* (2003), the force applied is related to the toughness of the samples, and how resistant the samples to shear. Higher firmness at the button stage showed higher endurance, and it would be exposed to minimal physical damage during postharvest handling since the structure is firmer. Meanwhile, at the mature stage, the veil was broken with the gills uncovered. They exhibited a fragile structure and can easily be fractured or torn. The mature stage of mushroom structure is more susceptible to metabolic damages, and highly perishable (Arumuganathan *et al.,* 2010; Kumar *et al.,* 2013).

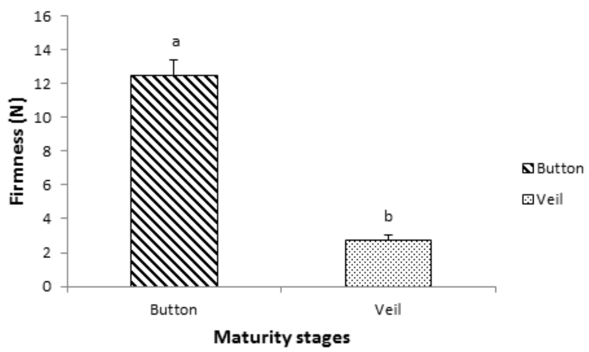

Figure 1. Firmness of *Volvariella volvacea* at button and veil opening stages.

The tissues during the mature stage are very fragile and easily fractured. This leads to consumers choosing button stage as it is more chewable and flavourful for consumption and cooking. This finding is in agreement with previous reports by Chang and Miles (2004), Ahlawat and Tewari (2007), and Jamjumroon *et al.* (2012) who stated that the button stage was the most practical stage to be picked and consumed. Also, the button stage of *V. volvacea* is more commercialised and sold in the market with premium price (Ahlawat and Tewari, 2007). In

Malaysia, farmers or sellers sold *V. volvacea* at MYR 15 – 20 / kg (USD 5 – 6 / kg).

Ultrastructure

Figure 2 shows the different structure of gills (lamella) at both maturity stages of *V. volvacea*. Based on Figure 2B, spores exist in tetrad for each of the basidium that contains a lot of sporangia. According to Chang and Miles (2004), the shape of these tetrad basidiospores are spherical, ellipsoidal, or egg-shaped. These could be clearly seen through SEM micrograph on the lamella surface of veil opening stage, and the sizes of mature spores were 6.33 ± 0.59 µm (Figure 2B). The spores were attached on the surfaces of the gills. However, they could not be seen by naked eye. The spores found at the gills are meant for reproduction including the meiosis process, karyogamy, and sporogenesis (Zied *et al.,* 2017).

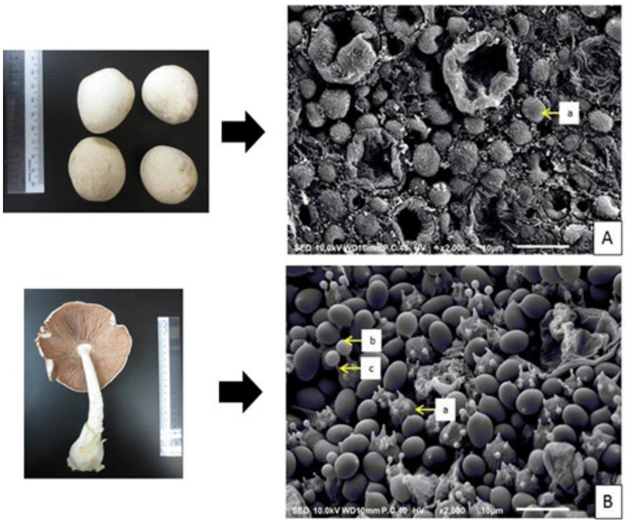

Figure 2. (left) Images of *Volvariella volvacea* after harvesting. (right) SEM micrographs of the surface structure of hymenium or lamella part at button stage (A), and veil opening stage (B) at 2000× magnification. a = basidium; b = basidiospore; and c = sterigmata.

According to Mohapatra *et al.* (2008), sporophore development contributes toward enzymatic concentration, and the browning reaction occurred in mushrooms. This is in agreement with Arumuganathan *et al.* (2010) who reported that mushrooms with exposed open gills were susceptible to higher perishability as compared to mushrooms with closed gills in veil. The metabolic activity would accelerate with the presence of spores.

The buttons (Figure 2A) showed no spores on their surfaces. The basidia started to form during button stage of about 3.83 ± 0.03 µm in length which enclosed the spores at later growth stages. The closed veil structure acts as membrane-type of epidermis that protects the mushrooms. Thus, it could benefit the postharvest management quality and commercialisation purposes for the mushroom. In addition, most consumers prefer the button stage for consumption (Chang and Miles, 2004; Ahlawat and Tewari, 2007; and Jamjumroon *et al.,* 2012).

Amino acid analysis

A total of 16 amino acids were found, which have been classified into essential and non-essential amino acids, in both immature (button) and mature (veil opening) stages of *V. volvacea*. All these amino acids are important in producing the flavour and taste of the mushroom (Johnsy and Kaviyarasans, 2015). The essential amino acids detected were histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine; while the non-essential amino acids were alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, hydroxyproline, serine, and tyrosine. Histidine and hydroxyproline were not detected in this mushroom.

Figure 3 shows that there was no significant difference in amount of amino acids at both stages. Nevertheless, there was a significantly higher

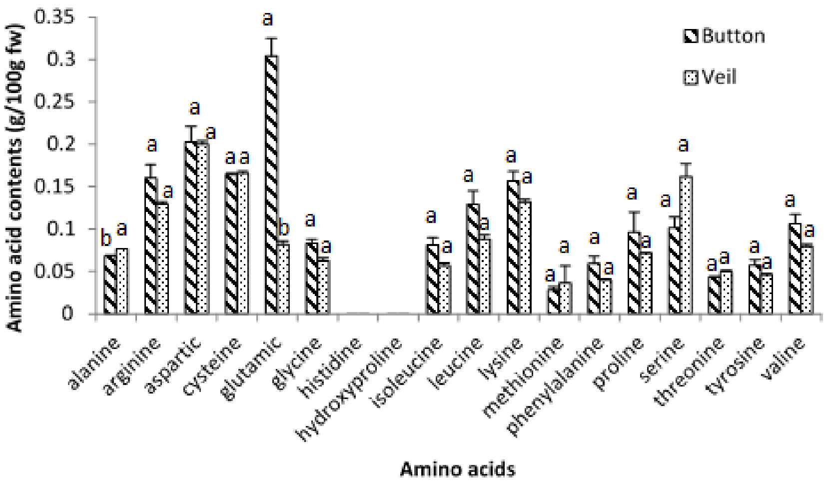

Figure 3. Amino acid contents (g/100 g FW) of button and veil opening stages of *Volvariella volvacea*.

percentage in alanine (11.11%) at the veil opening stage, and glutamic acid (73.19%) at the button stage. The result is in agreement with a previous study where the glutamic acid was the highest content as compared to other amino acids during button stage (Zakhary *et al.,* 1984). Although alanine was slightly higher in veil opening stage, it only contributes small amount of the total amino acids in the mushroom. Further, Zakhary *et al.* (1984) also reported the absence of glycine and isoleucine. In the present work, glycine and isoleucine were detected at very low amount of 0.083 and 0.081 g/100 g FW, respectively. None of the amino acid contents in this mushroom exceeded the EDI based on the values suggested by Dietary Reference Intake in g/100 g of amino acids. Therefore, the intake of these mushrooms is safe and can be consumed in higher quantity based on its amino acid contents.

Metals and health risk assessment

Heavy metals

Accumulation of heavy metals by mushrooms could be related to the natural and anthropogenic activities. Anthropogenic activities are the leading factors contributing to excessive bioaccumulation of heavy metals due to increase in industrialisation and urbanisation (Orisakwe *et al.,* 2012). While natural sources of metals occurred through the mushrooms' absorption from the soil or substrate they grow on (Sun *et al.,* 2017). The metals flowing throughout the food web might cause an inevitable challenge for people regarding their intake of mushrooms. Their non-biodegradable properties may cause the heavy metals long-lasting effect in the body, and eventually lead to adverse effects, as they exceed the standard permissible limit of metal intake (Tangahu *et al.,* 2011; Orisakwe *et al.,* 2012). Recently, mushrooms' tissues have become an indicator of the level of pollution to the environment (Tüzen, 2003; Sithole *et al.,* 2017). This is due to its properties of directly absorbing nutrients from its surrounding including metals (Rajapakse, 2011).

Table 1 shows no significant difference in heavy metals (Cu, Cd, Fe, Ni, Pb, and Zn) in the button and veil opening stages. However, *V. volvacea* demonstrates potential to absorb more metals from the EFB substrate. Therefore, as maturity increases, more metals accumulation will occur and could cause potential adverse effect to consumers as it is near the permissible safety limit. All essential heavy metals like Cu, Fe, and Zn yielded higher concentration, as compared to non-essential heavy metals like Cd, Ni, and Pb (Table 1). Thus *V. volvacea* can be a good source of compounds required for the human body. The presence of non-essential heavy metals could cause health effect toward the consumers. Nevertheless, excessive accumulation of essential heavy metals might also harm the human body due to its toxic effect (Ouzouni, 2009). According to Khani *et al.* (2017), accumulation of Pb in child caused degeneration in nervous system, thus attenuating their IQ and mind ability. While Cd could cause disturbances in human internal organs such as heart, bones, and kidney (Khani *et al.,* 2017), Ni is toxic, carcinogenic, and potentially damaging to the proteins and nucleic acids in the body (Kasprzak and Salnikow, 2007).

Estimated daily intake (EDI)

Table 2 shows the EDI of *V. volvacea* during the immature and mature stages. For essential heavy metals, Cu exceeded the recommended value set by FAO/WHO. Sithole *et al.* (2017) also reported that the Cu concentration was up to 51.30 mg/kg in *Agaricus bisporus* mushroom sampled from mining area. However, heavy metals such as Cd, Fe, Ni, Pb, and Zn did not exceed the permissible safety limit of standard heavy metal concentrations (Table 2). Cu, Fe, and Zn are vital for biological processes, while Cd, Ni, and Pb are highly toxic to the human body.

The slightly high Cu in the mushroom could be due to the absorption from the EFB substrate during growth, or its presence in the spawn media. Furthermore, the growth of mushrooms highly involves the absorption of nutrients from the surrounding (Kotwaliwale *et al.,* 2007; Rajapakse, 2011). The EFB substrate might be exposed to various environmental conditions with numerous background activity conducted during its plantation such as absorption from the soils, farming practices, and

Table 1. Heavy metal contents (mg/kg) of button and veil opening stages of *Volvariella volvacea*.

Maturity stage	Heavy metal					
	Essential			Non-essential		
	Cu	Fe	Zn	Cd	Ni	Pb
Button	70.89 ± 1.22[a]	151.49 ± 6.30[a]	62.14 ± 0.58[a]	0.11 ± 0.01[a]	6.16 ± 0.21[a]	0.54 ± 0.05[a]
Veil	73.13 ± 15.34[a]	174.20 ± 20.41[a]	81.87 ± 13.88[a]	0.11 ± 0.02[a]	6.48 ± 0.29[a]	0.49 ± 0.03[a]

Means with the same letter within a column are not significantly different at $p \leq 0.05$ using *t*-test (mean ± SE).

Table 2. Estimation of daily intake (EDI) and target hazard quotient (THQ) with recommended or permissible level of heavy metal intake via consumption per serving (300 g FW mushroom), by an adult consumer of assumption 60 kg average body weight.

Heavy metal		Button	Veil	Recommended (FAO, 1983; WHO, 1989)
Estimated daily intake (EDI) (mg/kg/day)				
	Cu	42.54 ± 1.27[a]	43.88 ± 15.95[a]	40.00
Essential	Fe	90.90 ± 3.78[a]	104.52 ± 12.25[a]	700
	Zn	37.29 ± 0.35[a]	49.12 ± 8.33[a]	300
	Cd	0.07 ± 0.01[a]	0.07 ± 0.01[a]	1.0
Non-essential	Ni	3.70 ± 0.12[a]	3.89 ± 0.17[a]	20
	Pb	0.33 ± 0.03[a]	0.30 ± 0.02[a]	4.0
Target hazard quotient (THQ)				
	Cu	0.39 ± 0.01[a]	0.40 ± 0.15[a]	
Essential	Fe	0.04 ± 0.00[a]	0.05 ± 0.01[a]	
	Zn	0.05 ± 0.00[a]	0.06 ± 0.02[a]	THQ < 1
	Cd	0.02 ± 0.00[a]	0.02 ± 0.00[a]	
Non-essential	Ni	0.06 ± 0.00[a]	0.07 ± 0.00[a]	
	Pb	0.03 ± 0.00[a]	0.03 ± 0.00[a]	

Means with the same letter within a column are not significantly different at $p \leq 0.05$ using *t*-test (mean ± SE).

emission of gases from transportation along the process until it became agricultural waste. Metal accumulation in mushrooms are also affected by pH and soil metal contents, organic matters, textures, and geographical areas (Sun *et al.,* 2017). However, the other metals showed far lower concentration of trace elements in both maturity stages of the mushroom. Thus, the intake of this mushroom is not deemed a worrisome or critical issue for human consumption. Furthermore, according to Roncero-Ramos and Delgado-Andrade (2017), an essential amount of edible fungi could help improve human health such as prevention from cancer disease, metabolic syndrome, obesity and hyperlipidaemia, hypercholesterolemia, diabetes, and hypertension.

Target hazard quotient (THQ)

Heavy metals receive a rising attention because of their ability to accumulate in the environmental components, and be deposited in plants (or in this case, mushrooms). Eventually, these accumulated metals will permanently occur in the food chain, and cause adverse health effect to human after consumption. These include disturbances in neurological systems that could lead to severe complications such as muscular dystrophy, multiple sclerosis, Parkinson's, and Alzheimer's diseases (Jaishankar *et al.,* 2014). Therefore, the examination of hazard risk index of produces should be taken into considerations.

Table 2 shows no significant difference was found on all the heavy metals value in terms of THQ at both the button and veil opening stages of the mushroom. All the THQ values of the heavy metals analysed were less than 1 (THQ < 1). The THQ value more than 1 could cause adverse health effects toward consumers (Sithole *et al.,* 2017). Therefore, this result showed that the consumption of this mushroom at immature and mature stages does not pose any adverse health effects and potential health risk toward human, based on the six metals analysed. However, continuous excessive consumption of this mushroom might cause adverse health problems in the later stages, especially on the nervous and other biological systems (Sithole et al., 2017). Previous study also reported THQ of less than 1 in *Pleurotus tuberregium* (oyster mushroom) (Ihugba *et al.,* 2018), *Agaricus bisporus* (white button mushroom) (Sithole *et al.,* 2017), and *Pleurotus florida* (white oyster mushroom) (Khani *et al.,* 2017).

Conclusion

In conclusion, significant differences in attributes measured between the button and veil opening stages of *V. volvacea* were not found as the gap of their stage developments was only about 12 h. The button stage was smaller in size, and contributed to the lower metabolic activity due to the absence of exposed gills-bearing spores as compared to that of veil opening stage. Besides, due to the firmer texture observed, the button stage of *V. volvacea* could fetch higher marketability (pleasure for consumer eatingexperience). In addition, the button stage of *V. volvacea* also showed a significantly higher content of glutamic acid. However, both button and veil opening stages of *V. volvacea* showed no significant differences in the total value of amino acids and heavy metals. For health risk assessment, EDI of *V. volvacea* consumption containing Cu, Fe, Cd, Ni, Pb, and Zn cultivated on EFB were within the permissible limit recommended by FAO/WHO. The Target Hazard Quotient ratio values for all metals studied did not exceed 1 (THQ < 1), which means that they did not pose any considerable health risk to the consumers at both maturity stages of *V. volvacea*.

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