Research Communication

Nutritional Evaluation on *Lignosus cameronensis* C. S. Tan, a Medicinal *Polyporaceae*

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Abstract

Sclerotial powder of a cultivated species of the Tiger Milk Mushroom, *Lignosus cameronensis* was analysed for its nutritional components and compared against species of the same genus, *Lignosus rhinocerus* and *Lignosus tigris.* All three species have been used by indigenous tribes in Peninsular Malaysia as medicinal mushrooms. Content of carbohydrate, fibre, mineral, amino acid, palatable index, fat, ash and moisture were determined. *L. cameronensis* sclerotial material consists of carbohydrate (79.7%), protein (12.4%) and dietary fibre (5.4%) with low fat (1.7%) and no free sugar. It has the highest content of total carbohydrate (791 g kg^{-1}), energy value (3,700 kcal kg^{-1}) and calcium (0.85 g kg^{-1}). The crude protein content (123 g kg^{-1}) is comparable to that of *L. rhinocerus* with its main amino acids consisting of glutamic acid, aspartic acid and leucine. The umami index is determined to be 0.27. The total essential amino acid (45 g kg^{-1}) is comparable to that of *L. tigris.* The main mineral is potassium (1.51 g kg^{-1}) and the Na/K ratio was <0.6. Heavy metals such as mercury, cadmium, lead and arsenic were absent. *L. cameronensis* has the highest amount of food energy, total carbohydrate and calcium compared to those of both *L. rhinocerus* and *L. tigris.* The essential amino acids comprised almost 40% of the total amino acid content, slightly more than that reported from sclerotial powder of the *L. tigris.* © 2019 IUBMB Life, 9999 (9999):1–6, 2019

Keywords: *Lignosus cameronensis*; nutrition; safety; sclerotia; tiger milk mushroom

INTRODUCTION

Tiger Milk Mushroom, *Lignosus* sp., belonging to the Polyporaceae family, is an important medicinal mushroom with the first record of its medicinal usage in Malaysia dating back to 1664 (1). The *Lignosus* mushroom in the wild is difficult to find, rare and often if found, would be growing in solitary, with not another known to be found within a radius of 5 km. Known members of this genus have been reported growing in Southern China, Africa, Southeast Asia, Papua New Guinea, New Zealand and Australia (2). Highly valued and popular among the indigenous communities of Peninsular Malaysia for its medicinal properties (3), this local mushroom, also known as "cendawan susu rimau," was believed to have sprung from the very spot where the lactate had fallen off of a nursing tigress. The sclerotium of *Lignosus* sp. is the part containing most of the medicinal properties compared to its fruiting body and has been used to treat asthma, fever, cough, cancer, food poisoning, wound healing and as a general tonic (1). It was not after continuous attempts of over 10 years that this wild mushroom, foremost *Lignosus rhinocerus*, could be cultivated by LiGNO™ Biotech Sdn Bhd and from then extensive research on the bioactive compounds and health-promoting properties of *L. rhinocerus* initiated (4). A number of biomedical properties of the aqueous extract of the *L. rhinocerus* were documented ever since and they include anti-inflammatory, neurite growth-promoting, antihypertensive, antiproliferative, antioxidant and enhancement of immunomodulatory activity (5–9).

Abbreviations: *AAS, atomic absorption spectrometer; AOAC, Association of Official Analytical Chemists; APHA, American Public Health Association; ASV, anodic stripping voltammetry; GFAAS, graphite furnace atomic absorption spectrometry; HCl, hydrochloric acid; HNO_3, nitric acid; ICP-MS, inductively coupled plasma-emission mass spectrophotometer; ITS, internal transcribed spacer; Sdn Bhd, Sendirian Berhad Private Limited (in English)*

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Following adequate studies and observation of *L. rhinocerus*, *Lignosus tigris* and *Lignosus cameronensis* were described as new species based on phenotypic and genotypic data collected from the two mushrooms from the tropical forests of Pahang (2, 10). Pore and basidiospore sizes are the main characters distinguishing various species, with *L. cameronensis* having basidiospores measuring 2.4–4.8 × 1.9–3.2 μm (10). The complete nuclear ribosomal RNA internal transcribed spacer (ITS) region consisting of ITS1 and ITS2 that flank the conserved 5.8S region discriminates *L. cameronensis* from the other *Lignosus* mushrooms (11). The ITS regions have been classified as good and reliable barcode genomic markers to identify and differentiate fungal species and lineages (12). It is therefore unclear if the indigenous communities or previous medicinal records would have correctly identified and described the *Lignosus* species used or collected for medicinal purposes. All three wild species could have been used to treat illness in the earlier days.

In terms of nutritional content, both cultivars of *L. tigris* and *L. rhinocerus* were reported superior to the wild types (2, 6). The sclerotia of *both cultivars* are richer in energy content, crude protein, macroelements with *L. rhinocerus* cultivar containing higher yield of all essential amino acids (6). Much of the carbohydrate in sclerotia of both species is starch (2, 6, 13). The closely related *L. cameronensis* with its origin from tropical forest in Lata Iskandar (4°17.46′N 101°34.41′E), Pahang, located over 1,000 m (3,300 ft) above sea level on the Titiwangsa Range (14) could contain similar nutrient composition. *L. cameronensis* like its other close relatives in the wild could have adapted to absorb, produce and store different amount of nutrients from poor soils for growth and reproduction. Since the sclerotia are mostly used instead of the fruiting bodies and the formation of fruiting bodies would have used up part of the nutrient reserves of the sclerotia, this study therefore accentuates to determine the nutrient composition in cultivated sclerotia of *L. cameronensis*, using similar optimally controlled growth (developmental) parameters for previous successfully cultivated *L. rhinocerus* and *L. tigris*. The profile of free amino acids will also provide us the umami index, flavour enhancer or sometimes described as the taste of protein, the "fifth" basic taste along with the traditional sweet, sour, salty and bitter tastes in culinary culture between Europe and Japan in recent years (15–17). The taste of medicinal mushrooms should be taken into consideration in the formulation of health food, as some mushrooms are bitter (18).

Earlier oral administration of sclerotial powder from all three species; *L. tigris* E, *L. rhinocerus* and *L. cameronensis* to Sprague Dawley rats in 28-day acute and sub-acute toxicity assessments showed no mortality or signs of toxicity (13, 14, 19, 20). As mushrooms collected in the wild could accumulate certain concerning levels of heavy metal pollutants (21, 22), an assessment on the level of heavy metals in cultivated fungal materials was also carried out in this study to provide solid evidence that the overall safety of cultivated *Lignosus* materials (all three species) is not compromised by the presence of heavy metals, which are tolerated only at very low safety levels in food and herbal medicine (23). This study adds new knowledge in the current literature regarding this new species of Tiger Milk Mushroom and evaluates its nutraceutical potential and safety as functional food.

EXPERIMENTAL PROCEDURE

Mushroom Material

The *L. cameronensis* mushroom has been confirmed its identity through ITS region via ITS-1 (5′-TT>GGCCCTT>CCCTT>CT GGCGG-3′) and ITS-2 (5′-GAGGCGCAGCCCTTCACTT-3′), (10, 11) which act as good and reliable barcode genomic markers for identification and differentiation of fungal species. It is mass cultivated by Ligno Biotech Sdn. Bhd. (Selangor, Malaysia) on sterilised substrate containing brown rice using proprietary formulation and left incubated in the dark at 24 °C for 3 months (duration for the formation of sclerotia) (14). The sclerotia were then harvested, without subjecting them to triggers that would lead to the next developmental stage of fruiting body. Sclerotia were randomly selected from different batches, processed, freeze-dried and milled into powder of 0.2 mm particle size.

Carbohydrate, Ash, Fibre, Fat, Protein, Sugar, Amino Acid, Mineral, Palatable Index and Moisture Analysis

Total carbohydrate, energy values and ash were measured using methods as previously described (24). The total carbohydrate value was estimated by subtracting the total of crude protein, total fat, moisture and ash from the total weight of the sample. The energy content was calculated through the amounts of protein, fat and carbohydrate in the sample the ash contents were obtained based on gravimetric weight loss (constant weight) after combustion of sample at 525–550 °C.

The values of total dietary fibre, soluble and insoluble fibres were measured using enzymatic gravimetric method of Association of Official Analytical Chemists (AOACs) (25) 985.29 and 991.43. Briefly, after fats were removed, the samples were treated with heat-stable α-amylase, protease and amyloglucosidase to degrade proteins and remove starch. The total dietary fibre was then obtained by subtracting the weight of proteins and ashes from the weight of precipitate. Total fat and crude protein content were calculated according to previously described methods (24, 26). The total fat was determined using a chloroform-methanol extraction-based gravimetric method. Crude protein content was determined using Kjedahl-based method with boric acid modification multiplying the total nitrogen content by the conversion factor 6.25. The sugar content was measured using AOAC 923.09 (25), based on the principle of Fehling's test. Titration was performed against Fehling's solution using standard sucrose and sample solutions respectively on heat. The presence of reducing sugar will reduce deep blue copper (II) complex of Fehling's solution to red precipitates of insoluble copper (I) oxide.

Amino acid content was analysed based on AOAC 994.12 (25). Performic acid oxidation was performed prior to hydrolysis to

oxidise cysteine and methionine to cysteic acid and methionine sulfone, respectively. Sodium metabisulfite was added to decompose performic acid. Amino acids were liberated from protein by hydrolysis with 6M HCl. Hydrolysates were diluted with sodium citrate buffer or neutralised, pH was adjusted to 2.2 and individual amino acid components were separated and measured via ion-exchange chromatography on amino acid analyser (Agilent) with ninhydrin post-column derivatisation.

Mineral and metal concentrations were determined based on APHA3120B, APHA3125, APHA3112B, AOAC 999.11 and AOAC 986.15 (25). Briefly, based on APHA3120B and APHA3125, 500 μL of sample was added to 5 mL of deionised water, vortexed until well mixed and atomized at high temperature in an inductively coupled plasma (ICP)-emission spectrometer coupled with mass spectrophotometer (MS). The ICP-MS would then scan, separate and quantify all ionised elements simultaneously. To detect mercury based on APHA3112B, the wavelength was set to 253.7 nm on a cold-vapour atomic absorption spectrometer (AAS). A standard curve was constructed by plotting peak height of standard from recorder chart versus a series of standard mercury solutions containing 0–5 μL. Mercury value of the sample was read from the standard curve. Determination of lead, cadmium, zinc, copper and iron was based on AOAC 999.11 (25) by dry ashing and flame atomic absorption spectrometry, flame and graphite furnace procedures. Samples were dried and ashed at 450 °C under a gradual increase (≤50 °C/h) in temperature. HCl (6 M) was added and the solution was evaporated to dryness. The residue was dissolved in 0.1M HNO_3 and the analytes were determined by flame and graphite procedures. The metals and elements were also determined using a multi-element method based on AOAC 986.15 (25). Sample solution was digested with HNO_3 overnight in closed system. Aliquots of digested sample solution were measured for cadmium and lead via anodic stripping voltammetry and arsenic, selenium and zinc via AAS.

The mushroom taste, umami or palatable index, is deduced from the ratio of aspartic and glutamic acids to total amino acids, taking the value within the range (0.21–0.32) (27, 28). Moisture content was measured using a moisture analyser. The sample's initial weight (5 g) was recorded and the sample was distributed evenly on the sample pan, infrared radiated and dried to a constant weight at 120 °C for 3 min. The moisture content was calculated as the total loss in weight.

All data presented are mean values of triplicate samples.

RESULTS

Carbohydrate, Protein, Moisture, Ash, Fat, Sugar and Fibre Content

The sclerotia contain 791 g kg^{-1} carbohydrate, 123 g kg^{-1} protein, 61 g kg^{-1} moisture, 8 g kg^{-1} ash and 17 g kg^{-1} fat (Table 1). Sugar was not detected. Insoluble fibre constituted 96.2% of the total fibre content which was at 54 g kg^{-1}.

TABLE 1 ***Carbohydrate, fibre, fat and ash analysis of L. cameronensis, L. tigris and L. rhinocerus (g kg^{-1})***

Composition	*L. cameronensis*	*L. tigris (11)*	*L. rhinocerus (6)*
Energy (kcal kg^{-1})	3,700	3,490	3,200
Crude protein	123	177	138
Total fat	17	17	8
Total carbohydrate	791	738	776
Fibre content	54	161	NA
Soluble fibre	2	8	NA
Insoluble fibre	52	153	NA
Total sugar	ND (<1)	56	30
Moisture content	61	60	NA
Ash	8	8	NA

Values determined by Association of Official Analytical Chemists (AOAC). ND, not detectable; NA, not available.

Amino Acid

Essential amino acids constituted 45.5 g kg^{-1} (40%) of the total amino acid content (114 g kg^{-1}) (Table 2). Glutamic acid, aspartic acid and leucine dominated all other amino acids at 17.4, 12.9 and 10.4 g kg^{-1}, respectively. Tryptophan was the lowest measuring amino acid at 0.454 g kg^{-1} along with cysteine, methionine, histidine and tyrosine measuring <4 g kg^{-1}. All other amino acids were more than 5 g kg^{-1}. No glycogenic amino acids such as hydroxyproline and hydroxylysine were detected. The umami index was 0.27.

Mineral and Heavy Metals

The highest essential mineral contained in *L. cameronensis* is potassium at 1.51 g kg^{-1} (Table 3), followed by calcium (0.85 g kg^{-1}), magnesium (0.53 g kg^{-1}), sodium (0.228 g kg^{-1}) and chromium (0.0001 g kg^{-1}). No measureable amount of toxic metals (lead, arsenic, mercury and cadmium) was detected.

DISCUSSION

The dry sclerotial powder of cultivated *L. cameronensis* contained a high amount of carbohydrate (79.7%), protein (12.4%) and dietary fibre (5.4%) but low in fat (1.7%). No free sugar was detected. The sugar absence and low amount of fibre suggest that the carbohydrate content of the sclerotia is mostly starch, like that being reported from sclerotia of cultivated *L. tigris* (2). *L. cameronensis* has, in addition, the highest amount of food energy, total carbohydrate and calcium compared to both *L. rhinocerus* and *L. tigris*. The sclerotia contained a total of 113.944 g kg^{-1} amino acid, with the abundant amino acids being glutamic acid (17.4 g kg^{-1}), aspartic acid (12.9 g kg^{-1}) and leucine (1.04 g kg^{-1}). The limiting amino acid in this study was tryptophan at 0.454 g kg^{-1}. The essential amino acids

TABLE 2 *Amino acids of L. cameronensis, L. tigris and L. rhinocerus (g kg^{-1})*

Amino acid (g kg^{-1})	*L. cameronensis*	*L. tigris (11)*	*L. rhinocerus (6)*
Glutamic acid	17.40	21.40	23.20
Aspartic acid	12.90	10.90	18.70
Leucine[a]	10.40	9.58	13.30
Alanine	7.53	7.77	11.40
Serine[b]	6.99	5.79	8.50
Proline[b]	6.94	6.04	1.70
Valine[a]	6.86	6.74	11.40
Arginine[b]	6.58	5.68	20.40
Lysine[a]	6.34	4.87	4.60
Threonine[a]	6.00	4.96	9.10
Phenylalanine[a]	5.94	5.87	8.50
Glycine[b]	5.02	5.59	8.90
Isoleucine[a]	4.59	4.77	9.30
Tyrosine[b]	3.87	3.42	6.50
Histidine[a]	2.74	2.81	3.40
Methionine[a]	2.16	2.34	3.90
Cysteine[b]	1.23	1.45	4.20
Tryptophan[a]	0.45	0.94	NA
Hydroxyproline	ND < 0.05	NA	NA
Hydroxylysine	ND < 0.05	NA	NA
Total amino acids	113.94	110.92	167.00
Total essential amino acid[a]	45.48 (39.9%)	42.88 (38.7%)	63.50 (38%)
Total semi-essential amino acid[b]	30.63 (26.882%)	27.97 (25.2%)	50.20 (30%)
Umami or palatable index	0.27	0.29	0.25

Values determined by Association of Official Analytical Chemists (AOAC).
ND, not detectable; NA, not available.
[a] *Essential amino acid.*
[b] *Semi-essential amino acid*

comprised almost 40% of the total amino acid content, slightly more than that reported from sclerotial powder of *L. tigris*. Hydroxyproline and hydroxylysine are specific amino acids of collagen, one of the abundant useful protein found in animals, and were found unexpectedly in several members of Mycota (29). *L. cameronensis* however did not contain collagen. All pulverised sclerotia possess umami indexes that were within the range of palatable mushroom taste. The potassium content of *L. cameronensis* is the lowest compared to the other two closely related *Lignosus* mushrooms (Na:K ratio <0.6) (30–32) but it is 60 times more than the sclerotia of another sclerotium-forming mushroom *Pleurotus tuberregium* in Africa (33). Diet which is rich in potassium but low in sodium may help to lower blood pressure (31) and adding *L. cameronensis* as a part of the diet would not tilt the balance of normal blood pressure.

Still, we have much to learn about Tiger Milk Mushroom in general. *L. cameronensis* appears to produce and store higher amount of food energy and carbohydrate compared to the other two species. More work will have to be carried out to validate and tie environmental stress and the ability or needs to build and store higher reserves of nutrient in sclerotia of *L. cameronensis*. Sclerotia of non-pathogenic fungi serve as resource-storage, survival and defence against abiotic and biotic assault: altitude, low temperatures, low nutrient, desiccation, insects, herbivores and microbes (bacteria and fungi) (34). The mechanism in which Tiger Milk Mushroom defends its nutrient-rich sclerotia thus warrants further research. Tetramic acids, indole diterpenoids, pyridines, diketopiperazines and 2,4,6-tri acetylenic octane diacid are among the secondary metabolites produced in sclerotia of fungi that act

TABLE 3 *Mineral and heavy metal compositions of L. cameronensis, L. tigris and L. rhinocerus (g kg^{-1})*

Composition	L. cameronensis	L. tigris (11)	L. rhinocerus (6)
Potassium, K	1.510	2.180	2.032
Calcium, Ca	0.850	0.234	0.193
Magnesium, Mg	0.530	0.436	1.479
Sodium, Na	0.230	0.012	0.088
Chromium, Cr	0.0001	0.0001	0.0001
Lead, Pb	ND (<0.001)	ND (<0.001)[a]	ND (<0.001)[a]
Arsenic, As	ND (<0.001)	ND (<0.001)[a]	ND (<0.001)[a]
Cadmium, Cd	ND (<0.001)	ND (<0.001)[a]	ND (<0.001)[a]
Mercury, Hg	ND (<0.001)	ND (<0.001)[a]	ND (<0.001)[a]

Values determined by Association of Official Analytical Chemists (AOAC). ND, not detectable.
[a] *Data generated alongside L. cameronensis.*

as their chemical defence system against fungivorous predators (35–37). Yet, from this study and previous studies with animals (3, 14), no accumulation of toxic elements and heavy metals were found in the cultivated sclerotia of *Lignosus* mushrooms. This makes cultivated *L. cameronensis*, *L. rhinocerus* and *L. tigris* safe materials, an important cornerstone of health food for human consumption.

CONCLUSIONS

L. cameronensis cultivar produces and stores higher energy compared to *L. tigris* and *L. rhinocerus*. The nutritional composition of *L. cameronensis* is similar to the other two mushrooms. All three Tiger Milk Mushrooms cultivars are free of heavy metals and safe for consumption. With the cultivation techniques established for all three species, more materials could be supplied for further bioactivity studies and extend our understanding of the medicinal potential and values of the mushrooms.

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AUTHORS' CONTRIBUTION

S. Y. Fung and N. H. Tan conceived, designed and carried out the experiment. C. S. Tan and S. T. Ng cultivated the *Lignosus cameronensis*. S. Y. Fung and P. C. H. Cheong analysed the data. S. Y. Fung and P. C. H. Cheong contributed to the writing of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests. C. S. Tan and S. T. Ng are associated with Ligno Biotech Sdn Bhd which cultivates and markets the mushroom.

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