

Chiang Mai J. Sci. 2021; 48(5) : 1236-1246 http://epg.science.cmu.ac.th/ejournal/ Contributed Paper

Nutritional Aspects of Three Termitomyces and Four Other Wild Edible Mushroom Species from Sri Lanka

Niranjan W. Gunasekara*[a], Chandrika M. Nanayakkara [a], Samantha C. Karunarathna [b] and Ravi L.C. Wijesundera [a]

- [a] Department of Plant Sciences, University of Colombo, Colombo 03, Sri Lanka
- [b] CAS Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, People's Republic of China

*Author for correspondence; e-mail: niranjan.wg@gmail.com

Received: 19 March 2021 Revised: 6 May 2021 Accepted: 13 May 2021

ABSTRACT

Species of Termitomyces are considered as a group of prime edible mushrooms owing to their unique meaty flavour and texture. Nutritional composition data of some species in this genus are scanty due to their rare and seasonal nature. On the other hand, the nutritional value of many Sri Lankan wild edible mushrooms remained unexplored. Therefore, proximate, mineral, fatty acid, amino acid, and free sugar compositions of T. eurrhizus, T. heimii, T. microcarpus, together with four other wild edible mushrooms (Auricularia sp., Lentinus squarrosulus, Pleurotus djamor and Schizophyllum commune) were investigated. Proximate and mineral compositions were examined using the Association of Official Analytical Chemists (AOAC) official methods. The fatty acid, amino acid and free sugar compositions were determined using chromatographic methods. Studied mushrooms showed 8.54-31.05% of crude protein, 1.21-12.35% crude fat and 22.81-69.13% available carbohydrate percentages on a dry weight basis (DW). The fraction of unsaturated fatty acids was over 60% of the total fatty acids and oleic acid was the major fatty acid. Total amino acid content varied between 0.80-6.87% DW and glutamic acid was the most abundant. Trehalose was the major sugar, and the content of glucose was insignificant. Species of Termitomyces showed the highest protein and lowest carbohydrate contents. Essential amino acids/total amino acid and essential amino acids/non-essential amino acids ratios of Termitomyces spp. were closer to the values recommended by FAO/WHO. Thus, Sri Lankan Temitomyces spp. demonstrated better nutritional properties compared to the other wild edible mushrooms included in the study.

Keywords: wild mushrooms, Termitomyces, nutrition composition, fatty acids, amino acids, free sugars

1. INTRODUCTION

Mushrooms have been recognized as healthy and nutritious food for thousands of years. A vast variety of wild mushrooms are found in Sri Lankan forests. Out of them approximately twenty-five species are considered to be edible and are occasionally consumed by local people. Among the edible mushrooms, species of *Termitomyces* are considered as a group of prime edible mushrooms due to their unique meaty flavour and texture.

Termitomyces mushrooms are widely spread throughout the tropical and subtropical regions in Asia and Africa. They grow inside termite nests of those belonging to the subfamily Macrotermitinae forming a symbiotic association [1-2]. Numerous attempts to cultivate Termitomyces spp. under artificial conditions have failed [1, 3]. Hence, they are harvested only from the wild and are highly valued due to their seasonal availability and difficulty in harvesting [1-3]. Studies of Termitomyces mushrooms are vastly delimited to the areas where they are readily available in the wild [2]. Termitomyces are particularly rich in proteins and micronutrients including vitamins such as ascorbic acid [2-3]. Termitomyces heimii and T. eurrhizus are two rare and seasonal species that are highly regarded by locals. Yet, limited data are available on their nutritional value [1, 3]. To the best of our knowledge, the fatty acid composition of T. eurrhizus and the amino acid composition of T. heimii have not reported earlier.

Moreover, the nutritional composition of Sri Lankan wild edible mushrooms has not studied in detail. Hence, this study aimed to report the proximate, mineral, fatty acid, amino acid, and free sugar compositions of three species of Termitomyces along with four other wild edible mushroom species native to Sri Lanka (T. eurrhizus, T. heimii, Termitomyces microcarpus, Auricularia sp., Lentinus squarrosulus, Pleurotus djamor and Schizophyllum commune). These species are spread throughout the country. Yet, they may seldom appear mostly during the rainy season in a variety of habitats ranging from home gardens to large forests. Species of Termitomyces can be found on the soil, while the other four species are found on decaying wood. Despite the fact that some of these mushrooms (except Termitomyces spp.) can be artificially cultivated, currently none of them are cultivated in Sri Lanka. Except from that, a sample of S. communae cultivated in a medium comprised of coconut leaves and coir dust was compared against its wild counterpart to investigate the effect of substrate on the nutritional composition.

2. MATERIALS AND METHODS

2.1 Mushroom Samples

Samples of Auricularia sp., L. squarrosulus, P. djamor, T. eurrhizus, and T. heimii were collected from wooded areas in Matara, Matale and Polonnaruwa districts of Sri Lanka during monsoon rains. Samples were washed with clean water to remove soil and debris and blotted dry. A cultivation media comprised of coconut leaves and coir dust, as described by reference [4] was used to obtain an artificially cultivated sample of S. commune. Taxonomic identifications of species were done using morphological characters [5, 6]. Homology search of 18S rRNA gene sequences of focal species was done using NCBI BLAST (https://blast.ncbi.nlm.nih.gov) to further confirm the identity of the species. Resulted sequences were submitted to GenBank and Genbank accession numbers are as follows: L. squarrosulus- KP982902, P. djamor - KP943504, T. eurrhizus - KP943505, T. heimii- KP943503, Auricularia sp.- KR907876, S. commune- KR706163 and T. microcarpus- KP780436. Voucher specimens were stored in the herbarium of the Department of Plant Sciences, University of Colombo, Sri Lanka.

2.2 Proximate and Mineral Composition

Proximate composition, moisture, ash, crude fat, crude protein, crude fiber and minerals (K, Ca, Mg and Fe) were analysed according to the AOAC official methods of analysis at the Department of Animal Science, University of Peradeniya [7]. Moisture content was determined gravimetrically by oven drying samples at 100 ± 5 °C to a constant weight. Other parameters were determined as follows; the percentage of ash by incineration at 600 °C, percentage of crude fat by extracting with petroleum ether using a Soxhlet apparatus, percentage of crude protein by the Kjeldahl method, percentage of insoluble fibre by the fritted glass crucible method, and percentage of minerals by atomic absorption spectroscopy. Nitrogen to protein conversion factor N*4.38 was used for the calculation of crude protein [8-11]. Available

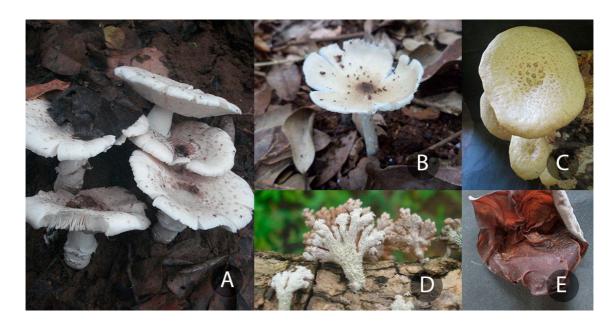


Figure 1. Some of the Sri Lankan wild edible mushrooms included in the study. Note: A: *Termitomyces heimii*; B: *T. microcarpus*; C: *Lentinus squarrosulus*; D: *Schizophyllum commune*; E: *Auricularia* sp.

carbohydrate was determined by the difference method excluding crude fibre [8]. Gross energy was calculated using the equation: Energy (kJ) = $4.184 \times (4g \text{ (protein)} + 3.75g \text{ (carbohydrate)} + 9g \text{ (fat)})$ [11].

2.3 Fatty Acids Composition

The fatty acid analysis was done according to the trans-esterification method using the gas chromatography with a flame ionization detector at the Industrial Technology Institute, Sri Lanka [9, 12-13]. One gram of mushroom powder was used to extract fat with petroleum ether using a Soxtherm automated fat extractor (Gerhardt, Germany) at 150 °C for 120 min. Extracted fat was derivatized with BF3/methanolic sodium hydroxide in iso-octane for 10 min at 60 °C. Fatty acid methyl esters (FAMEs) were extracted into hexane and evaporated to dryness. Shimadzu GC-2010 Gas chromatograph (Shimadzu, Japan) coupled with a flame ionization detector was used

for the detection of FAMEs. Separation of the fatty acid methyl esters was performed on a Supelco SP-2330 (30 m x 0.25 mm, 0.20 µm) capillary GC fused silica column using nitrogen (99.9999%) as the carrier gas with a pressure of 137.3 kPa at the injector port. The injection volume was 1.0 µl and the split ratio was 15:1. The separation was achieved using a gradient temperature program with an initial temperature of 100 °C and a final temperature of 220 °C in 50 min. Injector temperature and detector temperatures were maintained at 250 °C and 260 °C, respectively. GLC reference standard 461 (Nu-Chek Prep Inc., USA) with 33 FAMEs was used as the standard for the identification of FAMEs. Fatty acids were identified by comparing the retention time of chromatographic peaks with that of standard peaks. Relative percentages of fatty acids were calculated by internal normalization of chromatographic peak areas using LCsolution Ver.1 (Shimadzu, Japan).

2.4 Amino Acids Composition

Accurately weighed 0.5 g of dried mushroom powder was digested in a Mars Microwave Digester (CEM Corporation USA) for 20 minutes with 6 M HCl at 180 °C. Samples were neutralized and reconstituted up to 10.0 ml with double distilled water. The supernatant was filtered through 0.45 µm membranes before HPLC analysis. Determination of amino acids was performed according to the method described in Agilent ZORBAX Eclipse AAA Instructions for Use, Technical Note 5980-1193 at the Industrial Technology Institute, Sri Lanka [14]. Amino acids were detected using an Agilent 1260 Infinity HPLC (Agilent, USA) system equipped with a quaternary gradient pump, diode array detector and thermostatted column compartment. The separation was achieved on a ZORBAX Eclipse AAA ($4.6 \text{ mm} \times 150 \text{ mm}, 5 \mu \text{m}$) analytical LC column combined with a ZORBAX Eclipse AAA (4.6 mm \times 12.5 mm, 5 μ m) guard column. The mobile phase, solvent gradient and the HPLC method were used as instructed. Precolumn automated online derivatization of amino acids with o-phthalaldehyde was carried out before the detection under the UV at 338 nm (10 nm bandwidth), reference: 390 nm (20 nm bandwidth). The injection volume was 18.0 µl. The column compartment was thermostatted at 40 °C. The standard chromatogram was established using the amino acid standard mix, in 0.1 N HCl (Agilent, USA). Identification of amino acids was done by comparing the relative retention times of chromatographic peaks with the standard. Quantification of amino acids was achieved by calibration curves using OpenLAB CDS ChemStation Edition C.01.03 (Agilent, USA).

2.5 Free Sugar Composition

Free sugar composition was determined using HPLC system coupled to a refraction index detector at the Industrial Technology Institute, Sri Lanka [9, 15]. Dried and powdered mushroom samples (4 g) were defatted and then extracted with 40 ml of 1:1 mixture of water/ethanol at

40 °C. Solvents were evaporated, and the residue was reconstituted up to 1.0 ml with double distilled water and filtered using 0.45 µm membrane filters before HPLC analysis. The analysis was performed using an Agilent 1260 Infinity HPLC (Agilent, USA) equipped with a quaternary gradient pump, refractive index detector, and thermostatted column compartment. Separation of the free sugars was achieved with an Agilent ZORBAX Carbohydrate Analysis Column 4.6 mm × 250 mm, 5 µm coupled with an Agilent ZORBAX guard column 4.6 mm \times 12.5 mm, 5 μ m. Isocratic elution was carried out for 25 min with a flow rate of 0.8 ml/min using the mobile phase consisted of acetonitrile/ water (8:2 v/v). The injection volume was $5.0 \,\mu$ l. The system including the column compartment and the RI detector was thermostatted at 30 °C. Standard chromatograms were established using the D(-)-fructose, D(+)-glucose, D(+)-mannitol and D(+)-trehalose (Sigma Aldrich, USA). Identification of free sugars was done by the comparison of relative retention times of chromatographic peaks with the standards. Quantification of free sugars was achieved by calibration curves using OpenLAB CDS ChemStation Edition C.01.03 (Agilent, USA).

2.6 Statistical Analysis

All the analyses were done in triplicates and the results are shown as mean \pm standard deviation. The significant difference between the proximate and mineral composition of wild and cultivated *S. communae* samples was calculated using two sample t-Test assuming unequal variances (Microsoft Excel 2016) and means were considered significant at p < 0.05.

3. RESULTS & DISCUSSION

Sri Lankan wild edible mushrooms showed distinct proximate and mineral compositions, and the results are shown in Table1. Crude protein contents of our samples varied from 8.54% DW in *Auricularia* sp. to 31.05% DW in *T. microcarpus. Termitomyces* mushrooms had the highest crude

	Te	Tm	Th	Pd	Ls	As	Sc	Sc (ac)
Moisture*	92.08 ± 1.07	93.36 ± 0.53	92.79 ± 0.12	84.62 ± 1.14	90.35 ± 0.60	91.02 ± 0.11	91.26 ± 0.10	90.84 ± 0.02
Ash	11.52 ± 0.95	17.09 ± 1.17	13.58 ± 0.11	13.51 ± 0.51	7.45 ± 1.09	6.36 ± 0.73	3.95 ± 0.02	5.42 ± 0.15
Crude fat	6.27 ± 0.57	10.70 ± 0.96	12.35 ± 0.31	4.61 ± 0.53	2.31 ± 0.46	2.71 ± 0.62	1.21 ± 0.19	1.39 ± 0.33
Crude protein	29.40 ± 0.42	31.05 ± 0.58	28.54 ± 0.09	13.93 ± 0.75	11.68 ± 0.93	8.54 ± 0.09	14.41 ± 0.38	18.68 ± 3.86
Insoluble fibre	26.64 ±1.87	15.28 ± 0.42	22.72 ± 2.93	25.63 ± 3.15	26.38 ± 0.39	28.55 ± 1.77	11.09 ± 5.13	14.99 ± 3.71
Energy**	1138.70 ± 5.33	1328.51 ± 19.43	1300.57 ± 29.74	1070.87 ± 35.48	1100.85 ± 19.56	1089.60 ± 38.58	1376.44 ± 77.96	1298.85 ± 45.75
К	2.36 ± 0.11	3.08 ± 0.15	2.51 ± 0.15	3.01 ± 0.18	1.89 ± 0.19	1.38 ± 0.26	0.98 ± 0.02	1.30 ± 0.01
Mg	0.16 ± 0.01	0.05 ± 0.00	0.16 ± 0.01	0.15 ± 0.02	0.11 ± 0.05	0.08 ± 0.02	0.04 ± 0.00	0.12 ± 0.00
Ca	0.10 ± 0.00	nd	0.01 ± 0.00	0.02 ± 0.00	0.09 ± 0.00	0.06 ± 0.03	0.02 ± 0.00	0.08 ± 0.01
Fe	0.05 ± 0.00	nd	0.09 ± 0.00	0.28 ± 0.01	0.02 ± 0.00	nd	0.01 ± 0.00	0.03 ± 0.00

Table 1. Proximate and mineral composition (g/100g) on a dry weight basis of seven Sri Lankan indigenous mushrooms (mean \pm SD; n = 3).

Note: Te: *T. eurrbizus*; Tm: *T. microcarpus*; Th: *T. heimit*; Pd: *P. djamor*; Ls: *L. squarrosulus*; As: *Auricularia* sp.; Sc: *S. commune*; Sc (ac): *S. commune* (artificially cultivated); *calculated on a fresh weight basis. **average gross energy value kJ/100 g on a dry weight basis; K: potassium; Mg: magnesium; Ca: calcium; Fe: iron; nd: not detected.

protein content (28.54-31.05% DW) among all the studied samples. Previous studies have also shown higher protein contents in mushrooms [16-20]. And the percentage may vary between 19-35% DW in general for wild mushrooms [21]. Yet, except for the Termitomyces spp., crude protein content of our samples fell below the above range (8.54-14.41% DW). Moreover, the crude protein content of *T. heimii* was lower than the previously recorded value (34.2%) while in T. microcarpus crude protein content was slightly higher than previously recorded values (29.4-30.2%) [16, 20]. Wild mushrooms may contain about 2-6% DW crude fat [22]. Yet, the crude fat content of our samples varied in a comparatively broader range. Termitomyces spp. had the highest crude fat contents, and their crude fat percentages were higher than the previously reported values of 2.11% DW for T. heimii and 0.1-2.33% DW for T. microcarpus [16, 20]. Mushroom fibre is mainly comprised of β-glucans which shows beneficial effects as anticholesterol, anticancer, and immunomodulatory agents [23]. Data on the crude fibre content of wild mushrooms are scanty [22]. Yet a handful of studies report crude fibre contents between 4.78% DW in Macrolepiota rhacodes to 42.6% DW in Auricularia polytricha [18-19, 21, 24]. Our results were in agreement with the above values and ranged from 11.09% DW in wild S. commune to 28.55% DW in Auricularia sp. Five out of the seven samples contained more than 20% crude fibre, demonstrating them as a good source of fibre. Carbohydrate contents of wild mushrooms can be varied in a wider range from 6.4% DW in Armillariella mellea to 87.14% DW in Lentinula edodes [8, 11, 19, 22]. Similarly, carbohydrate values of our samples varied between 22.81% DW in T. heimii to 69.13% DW in wild S. commune.

The highest and lowest ash contents among our samples were found in *T. microcarpus* (17.09% DW) and wild *S. commune* (3.95% DW), while the highest and lowest mineral contents were detected in *P. djamor* (3.46 % DW) and wild *S. commune* (1.05% DW) respectively. Potassium is known as the major mineral in edible mushrooms [18-19, 22, 24]. Similarly, potassium (3.08-0.98%) was the most abundant mineral in all our samples. Calcium (0.01-0.10%) and Mg (0.04-0.16%) contents of our samples were compared with previously reported values for wild mushrooms [22].

Variations in crude protein, crude fat and crude fibre contents of studied species of T. heimii and T. microcarpus compared to the values reported in the literature may arise due to several factors such as intraspecific genetic variations of mushrooms in different geographical regions, developmental stage, part of the mushroom, pre-harvest and post-harvest conditions, time of harvesting, seasonal variations and storage conditions [9, 11, 21, 24]. Additionally, crude protein content can also be largely affected by the protein content of the growth substrate [23]. Moreover, we observed significantly higher (p < 0.05) percentages of ash, K, Mg and Fe in the cultivated S. commune sample compared to its wild counterpart which may have also resulted from the above factors. However, no significant difference (p > 0.05) was observed between two S. commune samples for other proximate nutritional parameters.

The fatty acid composition of Sri Lankan wild mushrooms expressed as the relative percentage of each fatty acid is shown in Table 2. One polyunsaturated, three monounsaturated and seven saturated fatty acids were detected. Percentage of monounsaturated fatty acids ranged from 13.85% in L. squarrosulus to 33.76% in Auricularia sp. The polyunsaturated fatty acid percentage varied between 30.41% in T. heimii to 59.09% in P. djamor. Thus, the total unsaturated fatty acid (UFAs) percentage was higher than the total saturated fatty acid percentage (SFAs) (26.40% in P. djamor to 38.64% in T. eurrhizus) for all studied samples. A higher percentage of UFAs over saturated fatty acids (SFA) in wild mushrooms also demonstrated in the literature [9, 24]. Unsaturated fatty acids hold vital physiological roles in the human body. Intake of polyunsaturated fatty acids (PUFAs) in place of SFAs improves the cholesterol profile by declining

the harmful LDL cholesterol. Consumption of MUFAs along with PUFAs such as linoleic acid in the place of SFAs is recommended as they may associate with reducing the risk of heart diseases [25]. The most abundant fatty acid was linoleic acid, ranging from 30.41% in T. heimii to 59.09% in P. djamor. It was followed by oleic acid (12.27%) in L. squarrosulus to 33.13% in Auricularia sp.) and palmitic acid (15.11% in Auricularia sp. to 26.4% in T. eurrhizus). The content of lauric acid was the lowest and it was found only in T. microcarpus (0.54%). Linoleic acid is considered an essential fatty acid and all our samples contained more than 30% linoleic acid in their fatty acid profile. The results obtained in the current study also agree with previous studies that showed linoleic, oleic, and palmitic acids as major fatty acids in most of the edible mushrooms including popular cultivated species such as Agaricus bisporus and Pleurotus ostreatus [9, 11, 17, 19, 24, 26].

The amino acid composition of our samples are presented in Table 3. Fifteen amino acids including seven essential amino acids (EAA) were found in almost all the samples. Glutamic acid was the major amino acid ranging from 124.8 mg/100g in P. djamor to 957.3 mg/100g in T. microcarpus. It was followed by aspartic acid (18.2 mg/100g in P. djamor to 574.1 mg/100g in T. eurrhizus) and glycine (64.8 mg/100g in P. djamor to 808.8 mg/100g in T. microcarpus). Leucine was the most abundant essential amino acid and its content varied from 39.7 mg/100g in P. djamor to 658.3 mg/100g in T. eurrhizus. The essential amino acid methionine was the most limiting amino acid in most of our samples and it was not detected in T. microcarpus and P. djamor. The highest total amino acid (TAA) content, 6.87% DW, was detected in T. eurrhizus while P. djamor had the lowest 0.80% DW. Our results were comparable with the previous studies that showed TAA contents varied between 2.85% (L. edodes) to 7.17% (Pleurotus eryngii) [27]. Moreover, previous studies have also demonstrated the presence of certain amino acids such as glutamic acid,

Fatty acid	Te	Tm	Th	Pd	Ls	As	Sc	Sc (ac)
Capric acid; C10:0 (SFA)	0.17 ± 0.01	nd	0.41 ± 0.04	0.39 ± 0.00	0.27 ± 0.05	0.84 ± 0.06	0.48 ± 0.02	3.20 ± 0.18
Lauric acid; C12:0 (SFA)	nd	0.54 ± 0.04	nd	nd	nd	nd	nd	nd
Myristic acid; C14:0 (SFA)	0.81 ± 0.03	0.22 ± 0.01	0.72 ± 0.02	0.47 ± 0.03	0.49 ± 0.03	0.66 ± 0.03	0.57 ± 0.07	2.21 ± 0.12
Pentadecanoic acid; C15:0 (SFA)	0.67 ± 0.01	0.20 ± 0.03	0.48 ± 0.00	2.02 ± 0.06	1.24 ± 0.14	1.34 ± 0.14	1.31 ± 0.11	1.62 ± 0.13
Palmitic acid; C16:0 (SFA)	26.40 ± 0.86	19.52 ± 0.32	22.09 ± 1.48	16.21 ± 0.45	25.48 ± 0.95	15.11 ± 0.61	18.10 ± 0.46	19.21 ± 0.45
Palmitoleic acid; C16:1 (MUFA)	3.97 ± 0.08	0.59 ± 0.05	1.29 ± 0.02	0.33 ± 0.02	0.98 ± 0.10	0.33 ± 0.02	0.78 ± 0.05	1.39 ± 0.08
Margaric acid; C17:0 (SFA)	0.94 ± 0.01	0.55 ± 0.06	0.91 ± 0.03	1.44 ± 0.21	1.27 ± 0.08	0.38 ± 0.02	0.70 ± 0.04	1.30 ± 0.06
Cis-10- heptadecenoic acid; C17:1 (MUFA)	0.81 ± 0.02	0.51 ± 0.00	0.54 ± 0.04	0.51 ± 0.04	0.60 ± 0.00	0.30 ± 0.0	0.53 ± 0.03	0.64 ± 0.06
Stearic acid; C18:0 (SFA)	8.78 ± 0.21	10.60 ± 0.85	12.31 ± 0.35	5.32 ± 0.32	4.97 ± 0.17	14.51 ± 0.34	5.31 ± 0.16	3.46 ± 0.13
Oleic acid; C18:1c, n-9 (MUFA)	20.22 ± 0.58	30.20 ± 1.17	30.23 ± 1.14	13.67 ± 0.43	12.27 ± 0.32	33.13 ± 0.93	14.46 ± 0.29	9.60 ± 0.54
Linoleic acid; C18:2c, n-6 (PUFA)	36.34 ± 1.59	36.70 ± 0.88	30.41 ± 1.64	59.09 ± 1.10	51.50 ± 1.37	30.96 ± 0.78	52.23 ± 1.97	52.13 ± 1.19
Arachidic acid; C20:0 (SFA)	0.87 ± 0.05	0.37 ± 0.03	0.63 ± 0.04	0.55 ± 0.05	0.94 ± 0.11	2.43 ± 0.24	5.54 ± 0.30	5.24 ± 0.24
SFA	38.64	32.00	37.55	26.40	34.66	35.27	32.01	36.24
MUFA	25.00	31.30	32.06	14.51	13.85	33.76	15.77	11.63
PUFA	36.34	36.70	30.41	59.09	51.50	30.96	52.23	52.13
UFA	61.34	68.00	62.47	73.60	65.35	64.72	68.00	63.76

Table 2. Fatty acid composition (%) of Sri Lankan indigenous mushrooms (mean \pm SD; n = 3).

Note: Te: T. eurrhizus, Tm: T. microcarpus, Th: T. beimit, Pd: P. djamor, Ls: L. squarrosulus, As: Auricularia sp.; Sc: S. commune; Sc (ac): S. commune (artificially cultivated); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; nd: not detected.

aspartic acid, glutamine, arginine and alanine as major amino acids, and methionine and cysteine as minor amino acids in edible mushrooms [11, 19, 22, 24, 28]. Essential amino acids to TAA percentage of *Termitomyces* samples varied between 34.29% in *T. microcarpus* to 38.52% in *T. heimii*. The ratio of EAA to non-EAA ranged from 0.52 in *T. microcarpus* to 0.63 in *T. heimii*. Those values closely resembled the reference values of EAA/TAA (40%) and EAA/non-EAA (0.6) recommended by FAO/WHO [29].

Content of glutamic acid and aspartic acid in mushrooms contribute to their unique flavour [22,

28]. According to the higher abundance, glutamic acid may act as one of the major umami taste components in edible mushrooms [21]. Based on the quantity of the umami taste components, foods are categorized as low (<5 mg/g), middle (5-20 mg/g) and high (>20 mg/g) umami taste foods [21]. Accordingly, mushrooms included in our study can be categorized in the middle umami taste category.

Even though hydrochloric acid hydrolysis remains the most widely used method for amino acid analysis in food, both releasing and destruction of amino acids take place simultaneously during this

Amino acid	Tm	Te	Th	Pd	Ls	As	Sc	Sc (ac)
Asp	410.4 ± 4.6	574.1 ± 4.0	465.4 ± 6.6	18.2 ± 0.2	524.7 ± 3.3	283.5 ± 3.0	423.8 ± 3.2	438.1 ± 3.5
Glu	957.3 ± 10.8	890.9 ± 14.2	587.9 ± 8.6	124.8 ± 4.4	754.1 ± 13.4	324.5 ± 4.2	541.0 ± 4.9	756.4 ± 9.2
Ser	325.9 ± 6.1	367.3 ± 2.7	152.5 ± 2.0	28.8 ± 1.0	196.1 ± 2.6	130.2 ± 1.4	253.2 ± 2.7	400.0 ± 2.7
Gly	808.8 ± 3.7	714.8 ± 11.1	315.8 ± 3.8	64.8 ± 0.8	388.9 ± 7.8	148.6 ± 1.3	235.2 ± 1.7	503.2 ± 7.7
Ala	571.9 ± 6.0	681.8 ± 9.2	322.0 ± 1.1	29.2 ± 0.5	385.9 ± 1.8	204.1 ± 3.2	334.5 ± 3.5	472.1 ± 6.5
Tyr	232.1 ± 4.4	198.9 ± 5.1	152.2 ± 1.3	nd	124.5 ± 2.2	80.0 ± 0.7	98.7 ± 0.7	180.4 ± 1.2
Arg (SE)	551.7 ± 4.5	582.9 ± 5.4	257.0 ± 1.9	58.2 ± 2.7	306.8 ± 4.7	178.5 ± 2.5	653.9 ± 4.4	825.4 ± 9.1
His (SE)	nd	312.3 ± 2.5	210.5 ± 3.6	292.2 ± 5.7	178.1 ± 2.0	121.7 ± 0.9	132.4 ± 0.6	294.9 ± 3.0
Thr (EAA)	203.7 ± 5.5	347.2 ± 1.5	180.9 ± 3.9	20.6 ± 0.6	197.2 ± 5.6	158.3 ± 2.1	161.8 ± 1.1	267.5 ± 1.5
Val (EAA)	337.0 ± 1.6	373.0 ± 7.3	265.3 ± 2.7	42.2 ± 0.4	343.3 ± 3.9	171.9 ± 1.6	160.7 ± 1.7	272.6 ± 2.3
Met (EAA)	nd	52.6 ± 2.3	58.3 ± 0.9	nd	253.1 ± 1.0	38.7 ± 0.9	57.7 ± 0.8	110.9 ± 0.7
Phe (EAA)	343.7 ± 3.1	366.1 ± 3.2	225.7 ± 2.5	31.4 ± 0.2	582.6 ± 6.3	165.9 ± 2.0	173.1 ± 2.0	274.9 ± 2.8
Ile (EAA)	229.8 ± 2.3	327.1 ± 2.5	204.2 ± 6.5	23.2 ± 1.1	293.2 ± 8.0	116.6 ± 1.7	112.2 ± 3.4	210.5 ± 2.6
Leu (EAA)	485.6 ± 10.1	658.3 ± 6.0	306.3 ± 3.2	39.7 ± 1.5	419.3 ± 6.6	212.1 ± 3.6	273.5 ± 2.9	530.1 ± 4.2
Lys (EAA)	413.1 ± 7.9	424.0 ± 4.2	302.4 ± 4.4	23.0 ± 0.7	343.6 ± 2.5	160.7 ± 2.1	271.0 ± 2.3	315.9 ± 2.0
Total EAA	2012.9	2548.3	1543.1	180.1	2432.3	1024.2	1210.0	1982.4
Total AA	5871.0	6871.3	4006.4	796.3	5291.4	2495.3	3882.7	5852.9

Table 3. Amino acid composition (mg/100 g) on a dry weight basis of Sri Lankan indigenous mushrooms (mean \pm SD; n=3).

Note: Te: *T. eurrbizus*; Tm: *T. microcarpus*; Th: *T. beimii*; Pd: *P. djamor*; Ls: *L. squarrosulus*; As: *Auricularia* sp.; Sc: *S. commune*; Sc (ac): *S. commune* (artificially cultivated); EAA: essential amino acid; SE: semi-essential amino acid; AA: amino acid; Asp: aspartic acid; Glu: glutamic acid; Ser: Serine; Gly: Glycine; Ala: Alanine; Tyr: Tyrosine; Arg: Arginine; His: Histidine; Thr: Threonine; Val: Valine; Met: Methionine; Phe: Phenylalanine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; nd: not detected.

process [30]. As a result, asparagine and glutamine may be completely hydrolysed into aspartic acid and glutamic acid. Serine and threonine can be destroyed by about 10% and 5%, respectively. Traces of impurities in acid induces the partial destruction of tyrosine. Tryptophan destroys during the acid hydrolysis process [30]. Cysteine requires to be oxidized into cysteic acid before determination while proline requires derivatization with 9-fluorenylmethyl chloroformate for its detection [14, 30]. Hence, we did not intend to quantify asparagine, glutamine, tryptophan, cysteine and proline in the present study.

Finally, we analysed the free sugar composition of our samples. The analysis was done for fructose, glucose, mannitol, and trehalose (Table 4). Trehalose

and mannitol were major sugars found in our samples while glucose and fructose were found in insignificant quantities. The highest and the lowest free sugar contents were detected in T. heimii (3.33% DW) and T. eurrhizus (0.06% DW) respectively. Our observations agreed with the literature data which show abundant quantities of mannitol and trehalose, and minute quantities of glucose, arabinose, and maltose in edible mushrooms [9, 11, 21-22, 24]. Mannitol and trehalose are known to play an important role in maintaining the firmness of fruit bodies and the volume growth of mushrooms [22]. Trehalose is also important in the translocation of carbon from mycelium to the fruit body [9]. Mannitol which is considered an active sweet component adds only a perception

Table 4. Free sugar composition $(g/100 g)$ on a dry weight basis of Sri Lankan indigenous mushrooms
(mean \pm SD; n=3).

	Tm	Te	Th	Pd	Ls	As	Sc	Sc (ac)
Fructose	0.03 ± 0.01	0.04 ± 0.01	0.16 ± 0.03	0.07 ± 0.01	0.10 ± 0.02	0.01 ± 0.00	0.05 ± 0.01	1.61 ± 0.07
Glucose	0.02 ± 0.00	nd	0.09 ± 0.02	0.02 ± 0.00	0.05 ± 0.01	0.12 ± 0.03	0.04 ± 0.00	nd
Mannitol	0.35 ± 0.04	0.02 ± 0.00	2.37 ± 0.26	0.80 ± 0.04	0.28 ± 0.03	nd	0.04 ± 0.01	0.51 ± 0.05
Trehalose	1.27 ± 0.07	nd	0.71 ± 0.09	0.92 ± 0.07	0.41 ± 0.03	0.34 ± 0.06	0.76 ± 0.05	4.83 ± 0.36

Note: Te: T. eurrbizus; Tm: T. microcarpus; Th: T. beimii; Pd: P. djamor; Ls: L. squarrosulus; As: Auricularia sp.; Sc: S. commune; Sc (ac): S. commune (artificially cultivated); nd: not detected.

of sweetness in mushrooms, it is not responsible for the unique taste of mushrooms [21].

4. CONCLUSIONS

Sri Lankan wild edible mushrooms included in our study contained 8.54-31.05% crude protein and were rich in unsaturated fatty acids and insoluble fibre. The most abundant mineral was potassium. Linoleic acid was the most abundant fatty acid in all the samples. Total amino acid content varied between 0.80-6.87% DW and glutamic acid was the major amino acid. Glucose content was negligible, and trehalose was the major sugar. No significant difference was observed between wild and cultivated S. commune samples for the studied proximate nutritional parameters, except for ash, K, Mg and Fe. Sri Lankan Termitomyces spp. contained a higher percentage of crude protein, crude fat, ash and insoluble fibre and a lower percentage of available carbohydrate compared to the other studied mushrooms. The fatty acid profile of T. eurrhizus indicated 36.34% PUFA, 25.00% MUFA and 38.64% SFA. Linoleic acid followed by palmitic acid and oleic acid presented as major fatty acids in T. eurrhizus. Amino acid profile of T. heimii revealed glutamic acid followed by aspartic acid and alanine as major amino acids, its total amino acid and total essential amino acid contents were 4.01% DW and 1.54% DW respectively. EAA/TAA and EAA/non-EAA ratios of studied Termitomyces spp. were closer to the values recommended by FAO/WHO. Thus, Sri Lankan *Temitomyces* spp. demonstrated better nutritional properties compared to the other mushrooms included in the study.

ACKNOWLEDGEMENTS

Authors acknowledge Professor H.W. Cyril, Department of Animal Science, University of Peradeniya, Sri Lanka for providing facilities to perform the nutritional analysis and S.S. Ediriweera, Department of Plant Sciences, University of Colombo for identification of mushroom samples and providing a sample of artificially cultivated *S. commune*.

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

FUNDING

This work was supported by the National Research Council of Sri Lanka under Grant NRC-11-040.

REFERENCES

- Rahmad N., Al-Obaidi J.R., Rashid N.M.N., Zean N.B., Yusoff M.H.Y.M., Shaharuddin N.S., et al., *Biol. Res.*, 2014; **47(1)**: 30. DOI 10.1186/0717-6287-47-30.
- Hsieh H.M. and Ju Y.M., *Appl. Microbiol.* Biotechnol., 2018; 102: 4987-4994. DOI 10.1007/ s00253-018-8991-8.

- [3] Malek S.N.A., Kanagasabapathy G., Sabaratnam V., Abdullah N. and Yaacob H., *Int. J. Food Prop.*, 2012; **15**: 809-814. DOI 10.1080/10942912.2010.506017.
- [4] Ediriweera S.S., Wijesundera R.L.C., Nanayakkara C.M. and Weerasena O.V.D.S.J., *Mycosphere*, 2015; 6(6): 760-765. DOI 10.5943/ mycosphere/6/6/10.
- [5] Coomaraswamy U., A Handbook to the Agarics of Sri Lanka, National Science Council of Sri Lanka, Colombo, 1979.
- [6] Coomaraswamy U. and Kumarasingham S., *A Handbook to the Macrofungi of Sri Lanka*, The Natural Resources, Energy & Science Authority of Sri Lanka, Colombo, 1988.
- [7] AOAC., Official Methods of Analysis, 16th Edn., Association of Official Analytical Chemists, Arlington, 1995.
- [8] Sanmee R., Dell B., Lumyong P., Izumori K. and Lumyong S., *Food Chem.*, 2003; 82: 527-532. DOI 10.1016/S0308-8146(02)00595-2.
- [9] Barros L., Baptista P., Correia D.M., Casal S., Oliveira B. and Ferreira I.C.F.R., *Food Chem.*, 2007; **105**: 140-145. DOI 10.1016/j. foodchem.2007.03.052.
- [10] Pereira E., Barros L., Martins A. and Ferreira I.C.F.R., *Food Chem.*, 2012; **130**: 394-403. DOI 10.1016/j.foodchem.2011.07.057.
- [11] Reis F.S., Barros L., Martins A. and Ferreira
 I.C.F.R., *Food Chem. Toxicol.*, 2012; **50**:191-197.
 DOI 10.1016/j.fct.2011.10.056.
- [12] ISO., Animal and Vegetable Fats and Oils -Preparation of Methyl Esters of Fatty Acids, 2nd Edn., ISO 5509:2000(E), International Organization for Standardization, Geneva, 2000.
- [13] AOAC., Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters, 991.39, Association of Official Analytical Chemists, Arlington, 2003.

- [14] Agilent Technologies, Agilent ZORBAX Eclipse AAA Instructions for Use 5980-3088EN, Agilent Technologies, Inc., Santa Clara, 2008.
- [15] Folkes D.J. and Jordan M.A., Mono and Disaccharides: Analytical Aspects; in Eliasson A.C., ed., *Carbohydrates in Food*, 2nd Edn., CRC Press, Boca Raton, 2006: 2-40.
- [16] Aletor V.A., *Food Chem.*, 1995; 54: 265-268.
 DOI 10.1016/0308-8146(95)00044-J.
- [17] León-Guzmán M.F., Silva I. and López M.G., J. Agric. Food Chem., 1997; 45: 4329-4332. DOI 10.1021/jf970640u.
- [18] Manjunathan J., Subbulakshmi N., Shanmugapriya R. and Kaviyarasan V., *Int. J. Biodivers. Conserv.*, 2011; **3(8)**: 386-388.
- [19] Sudheep N.M. and Sridhar K.R., *Mycology*, 2014; 5(2): 64-72. DOI 10.1080/21501203.2014.917733.
- [20] Thatoi T. and Singdevsachan S.K., Afr. J. Biotechnol., 2014; 13(4): 523-545.
- [21] Naknaen P., Itthisoponkul T. and Charoenthaikij
 P., J. Food Meas. Charact., 2015; 9: 259-268.
 DOI 10.1007/s11694-015-9231-x.
- [22] Kalač P., Food Chem., 2009; 113: 9-16. DOI 10.1016/j.foodchem.2008.07.077.
- [23] Carrasco-González J.A., Serna-Saldívar S.O. and Gutiérrez-Uribe J.A., J. Food Compos. Anal., 2017; 58: 69-81. DOI 10.1016/j. jfca.2017.01.016.
- [24] Guillamón E., García-Lafuente A., Lozano M., D'Arrigo M., Rostagno M.A., Villares A., et al., *Fitoterapia.*, 2010; 81: 715-723. DOI 10.1016/j.fitote.2010.06.005.
- [25] Harvard Health Publishing, The truth about fats: the good, the bad, and the in-between; Available at: https://www.health.harvard. edu/staying-healthy/the-truth-about-fatsbad-and-good
- [26] Harki E.I.H. and Ghanmi M., Moroccan J.

Biol., 2014; 11: 10-16.

- [27] Kim M.Y., Chung I.M., Lee S.J., Ahn J.K., Kim E.H., Kim M.J., et al., *Food Chem.*, 2009; **113**: 386-393. DOI 10.1016/j.foodchem.2008.07.045.
- [28] Ribeiro B., Andrade P.B., Silva B.M., Baptista P., Seabra R.M. and Valentão P., *J. Agric. Food Chem.*, 2008; 56: 10973-10979. DOI 10.1021/ jf802076p.
- [29] Zhou J. and Han D., J. Food Compos. Anal., 2006;
 19: 850-853. DOI 10.1016/j.jfca.2006.04.008.
- [30] Fountoulakis M. and Lahm H.W., J. Chromatogr.
 A, 1998; 826(2): 109-134. DOI 10.1016/ s0021-9673(98)00721-3.