



Proximate Composition, Minerals and Amino Acid Composition of a Two Edible Mushroom *Termitomyces microcarpus* and *T. heimii*

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ABSTRACT

Mushrooms are balanced food and good source of proteins, amino acids and minerals. The mushrooms were harvested fresh, sun dried, pulverized and analyzed according to standard procedure. Proximate analysis showed higher level of protein (30.43 and 26.77 %), crude fibre (9.09 and 10.83%), carbohydrates (34.35 and 33.3%), ash (9.1 and 7.1%), and low fat (3.27 and 2.11%). The mushroom was also found to contain variable amount of minerals. Mineral analysis of both species indicated the presence of potassium, sodium, magnesium, manganese, calcium, copper, zinc, phosphorus and iron. The most abundant component of the essential amino acid and non-essential amino acids were histidine (11.57 and 10.58mg/g) and glutamic acid (10.39 and 11.11 mg/g) are present in both species. Mushrooms are popular food all over the world. These findings showed that the mushrooms are the good source of nutritive food.

Keywords: Proximate analysis, *Termitomyces microcarpus*, *T. heimii*, minerals, amino acids.

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INTRODUCTION

Mushrooms have long been valued for their palatability and their use as food¹. Wild and cultivated mushrooms have been consumed by humans for their nutritional and medicinal benefits. There have been several studies cited in the literature highlighting the nutritive value of mushrooms from India². The consumption of wild edible mushroom is on the rise, even in the developed world and mushrooms have been considered as rich food because they contain protein, sugars, glycogen, lipids, vitamins, amino acids, crude fibers and mineral nutrients which are essential for normal functioning of the human body^{3,4,5}. Mushrooms are one of the highest protein producers per unit area and times. They can be recommended for the countries suffering from insufficient nutrition^{6,7}. Mushrooms have been found to accumulate heavy metals like cadmium, lead, arsenic, copper, nickel, chromium and mercury⁸. Interestingly, the mineral content of wild mushrooms have been found to be higher than the cultivated ones⁹. The uptake of minerals in mushroom is different from that in plants in many ways. Composition of trace elements and heavy metals is generally assumed to be species-dependent, but substrate composition is also considered to be an important factor^{10,11}. Amino acid composition is a reliable indicator of the nutritional value of food, including mushrooms¹². It is also believed that some amino acid contribute to the delicious taste of mushrooms, making them suitable for consumption. The chemical compositions vary depending on the species, variety, soil and other agro-ecological factors¹³. Based on the edibility of mushrooms, two wild edible Termitomyces species collected from the Western Ghats of Kanyakumari district to study their nutritional profiles.

MATERIALS AND METHOD

Mushroom collection

The wild edible mushrooms *T. microcarpus* and *T. heimii* were collected from the Keeriparai forest area of Western Ghats of Kanyakumari district, Tamil Nadu, India. It was collected during the rainy seasons, air dried and transported to the lab for analysis. The culture was maintained on a Potato dextrose agar medium and subculture every one month and the slants were incubated at 27°C for seven days and then stored at 4°C.

Proximate analysis

The samples were analyzed for chemical composition (moisture, protein, fats, carbohydrates and ash) using AOAC¹⁴ procedures. The crude protein content (N×4.38) of the samples was estimated by the macro-kjeldahl method; the crude fat was determined by extracting a known

weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at $600\pm 15^{\circ}\text{C}$. Total carbohydrates were calculated by difference.

Estimation of minerals

Mineral contents including Na, K, Fe, Zn, Cu, Cd and Pb were determined by atomic absorption spectrophotometer¹⁵. All the plastic and glassware were cleaned by soaking, with contact, over night in a 10% nitric acid solution and then rinsed with distilled water prior to use. The element standard solutions and HNO_3 used for digestion were supplied by Merck. A Perkin–Elmer Analyst 800 atomic absorption spectrometer with deuterium background corrector was used in this study. The operating parameters for working elements were set as recommended by the manufacturer. Lead and cadmium were determined by HGA graphite furnace using argon as inert gas. The other elements, iron, copper, zinc, potassium and sodium were determined by flame technique. After determining the ash content of the samples by oven method, ashes of the samples were dissolved in 100 ml nitric acid and kept in refrigerator until being analyzed. The element standard solutions were prepared by diluting stock solutions of 1000 ppm of sodium, cadmium, iron, zinc, copper, lead, potassium. The ratio of standard solutions prepared for each element, were different.

Estimation of Amino Acids

The amino acid composition was determined by high performance liquid chromatography (HPLC) based amino acid analyzer attached with a fluorescence detector¹⁴. The standard mixed chromatograms were established such as aspartic acid, glutamic acid, isoleucine, threonine, methionine, cystine, lysine, asparagine, glycine, arginine, valine, tryptophan, tyrosine, serine, leucine, phenylalanine, histidine, alanine, glutamine and proline. The test solution was prepared by dissolving the substance which was examined in the mobile phase for obtaining a concentration of 1.0 mg/mL. For the reference solution mixed amino acids Control Reference Standard (CRS) were dissolved in the mobile phase for obtaining a concentration of 1.0 mg/mL. The column was prepared by octadecylsilyl silica gel for chromatography R (3 μm) which acts as stationary phase. The size of the column should be $l = 0.10\text{ m}$, $\text{Ø} = 4.6\text{ mm}$. The stock solutions of 20 μl of test solution and standard solution of mixed standard amino acids were prepared by dissolving in double distilled water and then the mixture was constituted by mixing 1 mL each of the 21 standard amino acid solutions and this was later used to establish the standard chromatogram. For the mobile phase, 15.2g of triethylamine R was dissolved in 800 mL of distilled water and the pH was adjusted to 3.0 with phosphoric acid R and final volume was made-up to 1000 mL with distilled water. From this 850 mL of the solution was added to a

mixture of 2 volumes of propanol R and 3 volumes of acetonitrile R. The free amino acids in the standard and in *T. microcarpus* and *T. heimii* were automatically derived by reacting them with o-phthalaldehyde under basic conditions to produce o-phthalaldehyde derivatives in the reaction columns of the amino acid analyzer. Two derivative reagent solutions were prepared as follows: 10 mL of 0.01 M sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) buffer solution B (pH 9.1) were added to 10 mL of b-mercaptopropionic acid to make reagent solution I. Reagent solution II was prepared by mixing 10 mL of 0.01 M sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) buffer solution B (pH 9.1) with 10 mg of o-phthalaldehyde (OPA) dissolved in 3 mL of ethanol. Solutions I and II were filtered through 0.45 mm membrane filter before use. Following derivatization, the buffer solution A (mixed in acetonitrile in a 2:1 v/v ratio), containing the derivatized amino acid was transferred into the narrow bore HPLC system (HPLC column SRT ODSM, internal diameter = 4.6 and length = 150 mm) for separation at a temperature of 45°C with 20 μL injection volume and a flow rate volume of 1.0-1.5 mL/min. The detection was done using spectrophotometer at 220 nm and the run time was about 90 min.

Statistical analysis

Experimental values are given as means \pm standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA). Differences at $P < 0.05$ were considered to be significant¹⁶.

RESULTS AND DISCUSSION

Proximate analysis of *T. microcarpus* and *T. heimii*

The nutritive values are known to vary depending on the species, substrates, environmental conditions, age and part of fructification¹⁷. The dry matter content of fresh mushrooms is generally 5-15% and the nutritional profile of mushroom is directly affected by moisture content¹⁸. The moisture content of *T. microcarpus* and *T. heimii* lies in between the reference range 93.3% and 94.07% and the dry matter is 7.38% and 6.6% (Table 1). The high moisture content is an indication that fresh mushroom cannot keep for long time. Similar observations are made by Gbolagade *et al.*,¹⁹ for *Lycoperdon pusillum* containing moisture content 98.5% and dry matter is 1.5% and *Volvariella esculenta* showed 94.5% moisture and 5.5% dry matter. The major compounds present in mushrooms are proteins and carbohydrates. In the present study, it has been well proved that the test fungus *T. microcarpus* and *T. heimii* has high protein 30.43 % and 26.77% respectively. Ouzouni *et al.*,²⁰ reported that the protein content of the wild edible mushrooms *Hygrophorus chrysondon*, *H. russocoriaceus* ranged between 21.57 and 34.77% on

dry matter basis. In *Morchella deliciosa* contain 38.11% of protein at dry weight basis²¹. Mushroom proteins are generally higher than those present in green vegetables and fruits²². Vegetarians could also take mushrooms because it served as protein supplements in their diet.

Table 1: Proximate analysis of *T. microcarpus* and *T. heimii*

Parameters	<i>T. microcarpus</i>	<i>T. heimii</i>
Moisture %	7.38±0.55	6.6±0.92
Total ash %	9.1±1.311	7.1±1.25
Fat %	3.27±0.55	2.11±0.63
Protein %	30.43±1.6	26.77±3.37
Carbohydrates %	34.35±1.62	33.3±3.08
Fibre %	9.09±1.56	10.83±1.37

Data are expressed as mean ±SD (n=4)

Mushrooms are known to have a fairly large amount of carbohydrates, in the present study carbohydrates content 34.35% and 33.3% was recorded in the *T. microcarpus* and *T. heimii*. Florezak *et al*²³ reported that *Coprinus atramentarius* contains 24% of carbohydrate on a dry weight basis. Normal glucose and trehalose levels are low to the order of 100 g⁻¹ of dry matter. Soluble sugars in edible mushrooms were highly regarded as biologically or medically active compounds and were used as functional food ingredients or nutraceutical²⁴. The ash content varied between 9.1% and 7.1% in *T. microcarpus* and *T. heimii*. Barros *et al.*,²⁵ reported an ash content of 16.48 and 14.93 g/100g in the wild edible mushrooms such as *A. silvaticus* and *A. silicola* respectively. Nwanze *et al.*,²⁶ suggested that the crude fibre from *Lentinus squarrosulus* and *Psathyrella atroumbonata* provides a good source of dietary fibre for human consumption. However in the present study, the crude fibre content 9.09% and 10.83% was recorded in *T. microcarpus* and *T. heimii*. Jonathan *et al.*,²⁷ observed a crude fibre content of 6.41% in *L. squarrosulus*. Total dietary fibre in mushrooms is the sum of intrinsic non-digestible carbohydrates mainly, chitin²⁸. Crude fibre content of *T. microcarpus* and *T. heimii* was more when compared to the *L. squarrosulus* since anticholesterol effect was directly related to the crude fibre.

Mineral content of *T. microcarpus* and *T. heimii*

The mineral content of *T. microcarpus* and *T. heimii* is given in Table 2. The mineral concentration was determined on a dry weight basis. The amount of mineral contents in these organisms are related to species of mushroom, taxonomic position, collected site of samples, age of fruiting bodies and distance from the source of pollution or industrial site¹⁵. The mineral concentration in the mushroom are hardly affected by pH or organic matter content of the soil^{29,30}. Interestingly, the mineral content of wild edible mushrooms has been found to be higher

than the cultivated ones⁹. In compared with vegetables, mushrooms are a good source of many mineral elements eg the content of K, P, Zn and Cu varied in ranges 26.7-47., 8.7-13.9, 47-92 and 5.2-35 mg/kg (dw) respectively³¹.

Table 2: Mineral content of *T. microcarpus* and *T. heimii*

Minerals	<i>T. microcarpus</i>	<i>T. heimii</i>
Sodium (mg/g)	0.151±0.053	0.652±0.084
Phosphorus (mg/g)	0.634±0.10	1.268±0.29
Calcium (mg/g)	0.981±0.0433	0.929±0.043
Iron (mg/g)	0.378±0.039	0.334±0.01
Selenium (mg/g)	0.0063±0.002	0.004±0.001
Manganese (mg/g)	0.047±0.011	0.005±0.004
Copper (mg/g)	0.0098±0.0003	0.008±0.001
Chromium (mg/g)	0.0069±0.002	0.013±0.0004
Magnesium (mg/g)	1.188±0.15	1.36±0.16
Zinc (mg/g)	0.129±0.036	0.108±0.014
Potassium (mg/g)	2.422±0.43	1.337±0.11

Data are expressed as mean ±SD (n=4)

Composition of the trace elements and heavy metals is generally assumed to be species-dependent, but substrate composition is also considered to be an important factor¹⁰. The concentrations of minerals in shitake mushrooms are in general lower than those in cultivated white mushroom and in the oyster mushroom³². The differences in this regard could be due to the environmental variables, collection points and habitat and nature of the mushroom used²⁷. The trace element contents of the species depend on the ability of the species depends on the ability of the species to extract elements from the substrate, on the selective uptake and deposition of elements in tissue. The uptake of metal ions in macro fungi is higher than the plants³³. Good bioavailability of both copper and zinc from mycelium of *A. blazei* Murrill equating to very good levels of recommended daily intakes of these minerals from small amounts (1g) of this mushroom has been reported.

Amino acid content of the *T. microcarpus* and *T. heimii*

Amino acid composition is a reliable indicator of the nutritional value of food, including mushrooms³⁴. It is also believed that some amino acids contribute to the delicious taste of mushrooms, making them suitable for consumption¹³. The chemical compositions vary depending on the species, variety, soil and other agro-ecological factors. Mdachi *et al*³⁴ observed that *A. bisporus* and *P. ostreatus* are a good source of most amino acids. The most abundant component of the essential amino acid and non-essential amino acids were histidine (11.57 and 10.58mg/g) and glutamic acid (10.39 and 11.11 mg/g) respectively, are present in

both species. Amino acids analysis has shown that protein in a variety of mushrooms contain nutritionally useful quantities of essential amino acids, while tryptophan is a limiting amino acid in some varieties³⁵.

Table 3: Amino acid content of the *T. microcarpus* and *T. heimii*

Amino acids	<i>T. microcarpus</i>	<i>T. heimii</i>
Aspartic acid (mg/g)	7.55±1.19	7.64±1.15
Glutamic acid (mg/g)	10.39±0.89	11.11±0.51
Asparagine (mg/g)	6.51±0.91	3.93±0.62
Serine (mg/g)	4.06±0.55	2.81±0.47
Glutamine (mg/g)	3.15±0.56	3.89±0.91
Glycine (mg/g)	3.09±0.24	2.91±0.42
Threonine (mg/g)	2.65±0.63	2.69±0.54
Arginine (mg/g)	1.49±0.25	2.69±0.63
Alanine (mg/g)	4.108±0.18	3.54±0.5
Cystine (mg/g)	2.63±0.57	2.23±0.36
Tyrosine (mg/g)	5.39±0.55	3.45±0.3
Histidine (mg/g)	11.57±0.79	10.58±0.79
Valine (mg/g)	1.99±0.31	2.002±0.56
Methionine (mg/g)	11.56±0.59	11.96±0.96
Iso leucine (mg/g)	0.87±0.1	1.24±0.5
Phenylalanine (mg/g)	2.25±0.35	2.87±0.95
Leucine (mg/g)	2.78±0.66	3.72±0.71
Lysine (mg/g)	6.95±0.49	8.52±0.73
Proline (mg/g)	3.38±0.44	1.97±0.93
Tryptophan (mg/g)	4.57±0.45	3.75±0.91

Data are expressed as mean ±SD (n=4)

The most abundant essential amino acid and non-essential amino acid were leucine (0.61 g/100g) and glutamate respectively in *A. chaxingu*³⁶. Hong *et al*³⁷ reported that although several amino acids are found in *A. blazei*, glutamic acid was observed to be present at a higher level (41.37 mg g⁻¹). In *Agrocybe cylindracea* lysine and glutamate were the most abundant components of free essential and non essential amino acids¹³. In general, the differences between the observations made in this study and those of the others are assumed to be caused by differences in growth stages, harvesting time, methods and conditions of the experiments etc. The amino acid content is 15% in caps and 11% in stipes (dry matter). The amount of the amino acids phe, gly, his, arg and met are relatively higher than in *Agaricus* fruit bodies³². This agrees with Moore and Chi³⁸ that mushrooms has high nutritional attributes and potential applications in food industries.

CONCLUSION

These results suggest that mushrooms are very good nutritional source for mankind. *T. microcarpus* and *T. heimii* contains very poor lipid and very rich in protein, ash, fibre and

minerals. They could be very useful for vegetarians it contains more amounts of essential amino acids. Hence, *T. microcarpus* and *T. heimii* is comparable to the other edible mushrooms like *L. edodes*, *Agarius bisporus*, *Volvariella volvaceae* cited in the literature for its desirable composition and constitutes one of the functional, healthful foods. The present study demonstrated that *T. heimii* and *T. microcarpus* is a potential source of food due to its high carbohydrate content. In addition, the trace levels of toxic metals in this mushroom are within the safe level for consumption.

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