DOI: https://doi.org/10.36036/MR.32.1.2023.133845
***Mushroom Research* 32 (1) : 13-26, 2023**

Proximate composition, amino acids, fatty acids and mineral content of unexplored wild edible mushrooms of Odisha

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ABSTRACT

Wild edible mushrooms are considered as very good source of minerals and nutrients. The present study was conducted to evaluate proximate composition of macro and micro nutrients of nine most popular wild edible mushrooms of Odisha (*Termitomyces clypeatus, T. eurrhizus, T. heimii, Russula brevipes, R. nigricans*, *Lentinus fusipes, L. tuberregium*, *Volvariella volvacea, Tuber rufum*). The contents ranged from: Protein 0.80-20.53 mg/g, total carbohydrate 19.16-38.77g/100g, reducing sugar 5.55-16.25 mg/g, non reducing sugar 18.72-37.56 g/100g, ash content 3.63-7.36 g/100g, sterols 0-2.44 g/100g and total fibre 1.92-4.34 g/100g on dry wt basis. All the mushrooms contained large amount of free amino acids, fatty acids and vitamins. In addition to that the samples studied were exhibiting large amount of mineral nutrients too. The finding shows that the indigenous variety of mushrooms commonly palatable in Odisha can be used not only as a nutritious food but also for the pharmaceutical purposes.

Key words: *Termitomyces*, *Russula, Volvariella*, Amino acid, Fatty acid, mushrooms, vitamins, Edible mushrooms

Mushroom and other forms of macrofungi are in human consumption since ages. Edible mushrooms have been found in association with 13000 years old ruins in Chile. Reports of mushroom consumption appear in ancient writings, drawing, carvings of Roman empire, Chinese and Greek civilizations. The diversity of mushroom species is estimated to be as high as 1, 40, 000 but only about ten percents have been studied so far (Wasser, 2002). Fungi play a significant role in our daily life being utilized in industry, agriculture, medicine, food industry, textiles, bioremediation, as biofertilizers and in several other ways. The limitation in studying the mushroom in laboratory is an account of difficulty in culturing mushrooms artificially (Manoharachary *et al.,* 2005). Hence, attempts have been taken towards inventorization of unstudied microflora and /or fungi.

Mushrooms occur in various shapes, size and colour and grow in wide varieties of substratum i.e. soils, on decaying organic matter, wooden stumps, trees, rocks, marsh lands etc. More than 2000 species of edible mushrooms are reported from different parts of the world (Diez and Alvarez, 2001; Sanmee *et al.*, 2003). Mushrooms are gaining importance due to high nutraceuticals and pharmaceutical value. Many species of wild mushrooms are edible and are rich in essential nutrients such as carbohydrates, proteins, vitamins, fibres and several amino acids (Adedayo *et al.*, 2010). It is grown on commercial scale in many countries including India (Kansci *et al.*, 2003; Olila *et al.*, 2007; Oyetayo *et al.*, 2009). On account of its active ingredients, mushrooms are found to protect cardiovascular health, reduce cancer risk and can control blood sugar level, etc (Bobek, *et al.*, 1995;

Recieved on 27-02-2023; Accepted on 31-07-2023
https://epubs.icar.org.in/index.php/MR/article/view/133845

Konuk *et al.*, 2006). It's protective effects have been primarily attributed to the antioxidants properties of edible mushroom (Barros *et al.*, 2007; Barros *et al.*, 2008a; Barros *et al.*, 2008b). The nutritional quality of edible mushrooms is indicated by the quality, quantity and availability of proteins *in-vivo*. The amino acid and fatty acid components contribute to nutritive value of mushrooms (Guzman *et al.*, 1997; Ogundana and Fagade, 1982; Senetore, 1990; Thimmel and Kluthe, 1998). In view of amino acid composition mushrooms are also compared to animal proteins (Finks and Hoppenhaus, 1958; Gruen and Wong, 1982). Besides having nutritional quality, mushrooms have the potential of accumulating heavy metals, pesticides and radioactive substances (Konuk, *et al.*, 2007) signifying its phytoremediation attributes.

The presence of vitamins like riboflavin, biotin and thiamine are commonly reported in mushrooms (Atri *et al.*, 2012). Due to nutritive and delicious flavour and texture the search for wild edible mushrooms that can be commercially grown has recently increased. A number of studies have therefore been conducted on the nutritional and biochemical composition of edible mushrooms from different countries, such as Spain (Diez and Alvarez, 2001), Italy (Manzi *et al.*, 2001), India (Agahar-Murugkar and Subbulakshmi, 2005); Nigeria (Aletor, 1995) and Portugal (Barros *et al.*, 2007). The Indian subcontinent is known for its varied agro climatic zones with a variety of habitats that support rich mushroom diversity. About 850 species of macrofungi are recorded from India (Deshmukh, 2004). Surveys in the Himalayan region have been compiled by Lakhanpal (1996), records from Punjab, Kerala and Western Ghats have been published during last few years (Atri *et al.*, 2002). Longvah and Deosthale (1998) conducted nutritional studies on wild edible mushrooms from North East India. Agahar-Murugkar and Subbulakshmi (2005) studied nutritional value of edible wild mushroom of Khasi hills of Meghalaya. Johnsy *et al.* (2011) reported on the nutritive value of wild mushrooms collected from Western Ghats of Kanyakumari. Prakasam *et al.* (2011) studied the *Tricholoma giganateum* from southern part of India.

Mushrooms need to be explored more to study varietal difference in their nutritive and pharmacological value in order to identify suitable edible mushroom for commercial farming and to popularize the species amongst farming community.

Mushrooms are also favourite wild foods of tribals of Odisha and provide nutritive value to diet of poor which mainly consists of carbohydrates and is low in proteins and vitamins. Hunger and food insecurity persists in forested tribal region of Odisha. Considering lack of studies on mushrooms in Odisha state, an extensive survey in five forested and tribal districts i.e, Mayurbhanj, Keonjhar, Sundergarh, Kalahandi and Koraput districts was taken up. Odisha forest has rich floral diversity viz., Northern tropical semi evergreen forest, Northern tropical dry deciduous forest, Secondary moist miscellaneous semi evergreen forest, Mixed forests having combination of Northern tropical semi evergreen forest as well as Tropical moist deciduous forest and Tropical dry deciduous forest. It has a subtropical monsoon and humid climate; average rain fall in forest is 1500 mm' temperature ranges from 20-38 °C and humidity ranges from 58-88%. In addition to inventorization and characterization of mushroom species from different forest divisions of Odisha, market surveys were also conducted to identify edible species collected from wild by local inhabitants.

In the present study, we evaluated the nutritional and biochemical composition of common and marketed wild edible mushrooms of Odisha. Nine edible mushrooms (*Termitomyces clypeatus, Termitomyces eurrhizus, Termitomyces heimii, Russula nigricans, Russula brevipes, Lentinus fusipes, Lentinus tuberregium, Volvoriella volvacea, Tuber rufum*) were analyzed for dry

matter, proteins, fat, carbohydrate and ash content. Among the individual components, fatty acid, amino acid, vitamins profiles were investigated. The report regarding the nutritional and biochemical profiling in the present analysis is the first report in the species selected in the present context, though Singdevsachan *et al.* (2012) reported some components in an other species.

MATERIALS AND METHODS

Sample collection

Healthy, fresh and succulent mushrooms of *T. clypeatus, T. eurrhizus*, *T. heimii, R. nigricans*, *R. brevipes, L. fusipes*, *L. tuberregium*, *V. volvacea* and *T. rufum* were collected from tropical moist deciduous and semi ever green forests and stored in dried form. Macroscopic and microscopic examination of pileus, stipe, veil, ring, volva, lamellae and gills, etc. were made to identify species following procedure of Largent (1981). In addition to that, mushroom samples were identified from the ARI Pune, for authenticity. All the assays were performed using the entire mushroom fruiting body including stipe. A fine dried mushroom powder (100 mesh) was prepared for each species and used for biochemical analysis. The mushroom samples were analysed in the Microbiology laboratory of Regional Plant Resource Centre, Bhubaneswar, Odisha, India.

Estimation of mineral content

500 mg of sample of each mushroom species was weighed and kept in 10 mL concentrated Nitric acid overnight. The mixture was heated till the emergence of white fume and treated with diacid (Hydrochloric acid and Perchloric acid in 2:1) with the help of Microwave digestion system (Milestone stat D, Germany).The sample volume was made up to 100 ml with distilled water. Estimation of Calcium and Magnesium was done by titration method taking EDTA as titrate (Hesse,1971). Estimation of Phosphorous was done by using Vandatemolybdate and Orthophosphoric acid giving yellow colour complex in Nitric acid medium (Bray and Kurtz, 1945). Estimation of Sodium was done by flame photometry and estimation of Zn, Cu, Co & Cr was done by atomic absorption spectrophotometer (Analytic Jena, Germany) (Tandon, 1999).

Proximate analysis

The protein content was determined from the dried powder of mushroom (100 mesh size). One gram of dried powder of mushroom was treated with 10 mL of phosphate buffer (pH 7.6) and centrifuged for 20 min at 8000 rpm at 20 °C. 100 µL of supernatant was mixed with 5 mL of Bradford reagent, incubated in dark (10 min.) and absorbance was recorded at 595 nm. The protein values were expressed in mg per gram of dried sample (Bradford, 1976).

The ash content was analyzed by weighing the sample before and after burning at 500 °C overnight (17 h). Sterol content was measured by HPLC, taking chloroform and hexane as solvents (Zhang *et al.*, 1999). HPLC analysis was carried out at ambient temperature on a column (0.45 x 15 cm). Flow rate was 0.6 mL/min and elute was monitored by a UV-detector at 208 nm. The mobile phase was an isocratic mixture of acetonitrile and 2-propanol (4: 1) and the flow rate was 0.60 mL/min.

The total dietary fibre from the mushroom was determined according to Lee *et al.* (1992). Estimation of total carbohydrate was done by phenol sulphuric acid method. Glucose was taken as standard and the readings were taken at 490 nm (Dubois *et al.*, 1956; Hedge *et al.*, 1962). Estimation of reducing sugar was done by following dinitrosalicylic acid method (Miller, 1972) . Non reducing sugar was calculated by subtracting total carbohydrates and reducing sugar. The value was expressed in g/ 100 g dry weight of the sample (Nazarudeen, 2010).

Estimation of niacin and riboflavin was done spectrophotometrically (Sadasivam and Manickam, 1996). Estimation of Vit-D was done by HPLC method where filtered mixture of n-hexane and isopropyl alcohol (99:1) was taken as mobile phase. The liquid chromatograph was equipped with a 265-nm detector and a 4.6-mm × 15-cm column that contained 3-µm packing L8. The flow rate was about 1 mL/ minute and the relative standard deviation for replicate injections was not more than 3.0%. For the estimation of Thiamine the liquid chromatograph was equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contained packing L1. The flow rate was about 2 mL/minute. Pyridoxine in the mushroom sample wasquantified by the liquid chromatographywhichwas equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contained packing L1. The flow rate was about 1 mL/ minute. Chromatograph the Standard preparation, and record the peak areas as directed. The relative retention times of pyridoxine was about 0.5; and the relative standard deviation for replicate injections was not more than 3.0% (Anyakora *et al.*, 2008; Byrdwell *et al.*, 2011).

For the estimation of folate the column elutes was monitored with detector at 282 nm. The mobile phase was 0.1 mol. L-1 KH_2PO4 (pH 7.0), methanol, 90:10. The mobile phase was filtered through membrane at 0.5 µm. The flow rate was 0.7 mL/min. The column was operated at room temperature (Rahimia and Goodarzib, 2011).

Analysis of amino acid was done by using HPLC based amino acid analyzer by following the method described by Chirinang and Intarapichet (Chirinang and Intarapichet, 2009). Mobile phase was prepared by dissolving about 15.2 g of triethylamine in 800 ml of water; pH 3.0 was adjusted with phosphoric acid and diluted upto 1000 ml with Millipore water (Merck). 850 mL of this solution was added to 150 mL of a mixture of 2 volumes of propanoland 3 volumes of acetonitrile. Dissolved the mushroom sample to be examined in the mobile phase to obtain a concentration of 1.0 mg/mL octadecyl silyl silica gel for chromatography was served as a Stationary phase (3 µm).Flow rate was 1.0-1.5 mL/min. 20 µl of test solution /Standard was used for injection with run time of 90 minutes.

For fatty acid analysis the test solution was treated with butyl hydroxyl toluene and passed through nitrogen gas and being simultaneously added with required volume of Sodium hydroxide and methanol and sealed with poly tetrafluroethylene. After heat treatment, trimethylpentane was added and vortexed for 30 min. The clear upper layer was transferred to another tube after treatment with saturated sodium chloride and the methanolic layer was washed twice with trimethyl pentane. The combined extract was washed with water and dried in anhydrous sodium sulphate. Fused silica gel was used as column material. Stationary phase was taken as macrogol 20 000 R (film thickness 0.25 µm). Hydrogen was used as carrier for gas chromatography. The identification and quantitation of fatty acids were performed by gas chromatography using an Agilent 6890 series GC systemors. In analysis, GC gas flow rate was 1.3 mL/ min and injection volume was 1 µL. The temperature in the column was maintained at 50 ºC a minute, then the first temperature gradient of 10 ºC/min to 230 ºC for 5 min, the finally gradient temperature was 3 ºC/ min to 270 ºC and held for 8 min. Injector temperature was 250 ºC (Yilmaz *et al.*, 2013).

RESULTS

Proximate analysis

The proximate composition of nine edible mushrooms on fresh weight basis is shown in Table 1. The protein content of the studied mushrooms varied widely ranging from 0.08 to 2.05 g/100g. *T. eurrhizus* and *L. tuberregium* were rich in protein and showed higher amount of protein. Amongst sampled specimens the ash content ranged from 3.63

Table 1. Proximate chemical composition (on fresh weight basis) (±SD, n =3)

Parameters (g/100g)	*T. clypeatus*	*T. eurrhizus*	*T. heimii*	*R. nigricans*	*R. brevipes*	*L. fusipes*	*L. tuberregium*	*V. volvacea*	*T. rufum*
Protein	1.41±0.06	2.05±0.09	0.49±0	0.08±0.02	0.24±0.01	1.13±0.02	1.58±0.21	1.02±0.02	0.09±0.01
Carbohydrate	19.16±5.76	23.61±4.68	21.05±4.46	22.88 ± 2.43	27.38±3.71	37.31±4.17	30.05 ± 12.29	27.05±1.81	38.77±4.06
Red. Sugar	0.56±0.18	1.63±0.58	0.76±0.22	0.75±0.14	2.12±0.28	0.93±0.23	1.18±0.19	1.24±0.18	1.15±0.19
Non red. Sugar	18.60±5.58	21.98±4.10	20.28±4.24	22.13 ±2.29	25.26±3.43	36.38±3.94	28.87±12.10	25.81±1.62	37.61±3.87
Ash content	5.49±0.14	7.36±0.272	7.32±0.18	3.63±0.32	6.59±0.25	4.82±0.21	5.60±0.22	4.62±0.21	5.44±0.09
Sterols	Nil	2.41±0.32	2.44±0.20	Nil	Nil	Nil	Nil	Nil	Nil
Total fibre	3.16±0.04	3.84±0.11	4.34±0.08	1.926±0.05	3.57±0.33	3.65±0.10	2.56±0.10	3.78±0.23	3.50±0.07

to 7.36 g/100g and *T. eurrhizus* and *T. heimii* exhibited relatively higher ash content. Sterol content was minimal and recorded only in two species, i.e., *T. eurrhizus* and *T. heimii*. The carbohydrate content ranged between 19.16 g/100g and 38.77 g/100g. Maximum value was found in *T. rufum* (38.77 g/100g followed by *L. Fusipes* 37.31g/100g). Comparatively, *R. brevipes* had high amount of reducing sugar (2.12 ± 0.28 g/100g) than other edible mushrooms studied. *L. tuberregium, V. volvacea* and *T. rufum* have more or less similar amount of reducing sugars ranging between 1.15 ± 0.18 to 1.24 ± 0.18 g/100g. On the other hand, very high amount of non reducing sugar was analyzed in *T. rufum* (37.56 ± 4.07 g/100g) and *L. fusipes* (36.31±4.46 g/100g).

All mushrooms exhibited presence of the crude fibre (1.92 – 4.34 g/100g). Most mushrooms have similar crude fibre content except *R. nigricans* which contained least quantity (1.926 ± 0.05 g/100g) and the highest being in *T. heimii* (4. 34 ± 0.08 g/100g).

Minerals

The elemental concentration of the studied species on dry weight basis is given in Table 2. Although analysis of sample was done for selenium as well but no significant amount was detected. Highest amount of K was found in *T. rufum* (1.45 %) followed by *R. brevipes* (1.21 %). Amongst the edible ones *T. eurrhizus* was observed to have very high Iron content (5975.00 ppm), almost double the amount

Table 2. Mineral contents of wild edible mushrooms (on dry weight basis)

Species	P (%)	Na (%)	Ca (%)	Mg (%)	K (%)	Fe (ppm)	Zn (ppm)
Termitomyces clypeatus	0.23	0.06	11.40	9.42	1.12	2609.00	4.10
Termitomyces eurrhizus	0.25	0.09	22.12	11.22	ND	5975.00	0.90
Termitomyces heimii	0.32	0.15	21.08	12.02	ND	1773.50	4.10
R. nigricans	0.23	0.15	0.98	0.03	1.15	2950.33	68.66
Russula brevipes	0.28	0.13	16.40	10.50	1.21	2373.00	2.02
Lentinus fusipes	0.20	0.03	0.32	0.07	1.09	3998.00	45.00
Lentinus tuberregium	0.30	0.16	30.60	10.52	1.04	1055.00	2.20
Volvoriella volvacea	0.87	0.17	0.94	0.20	1.09	573.33	130.33
Tuber rufum	0.33	0.11	20.92	10.50	1.45	3021.50	40.40

recorded for other species i.e., *R. nigricans* (3998 ppm) and *T. rufum* (3021.50 ppm). *V. volvacea* contained very high amount of phosphorus (0.87 %) and Zn (133.33 ppm). A varying level of magnesium was noted in wild varieties (0.03-12.02 %). Maximum was recorded in *T. heimii* (12.02 %) followed by *T. eurrhizus* (11.22 %). The range for sodium was high (0.03-0.17 %), highest being in *L. tuberregium* (0.16 %) and *V. volvacea* (0.17 %). Mineral analysis of *L. tuberregium* showed high level of calcium content (30.60 %) whereas the Ca content was almost uniform for *T. eurrhizus* (22.12 %), *T. heimii* (21.08 %) and *T. rufum* (20.90 %). All mushroom tested were found to be good source of Zn except *R. brevipes* and *L. tuberregium*.

Amino acid composition

The result of free amino acid compositions on dry weight basis is given in Table 3. A total of 20 amino acids have been analyzed from the dry mushrooms samples. The concentration of free amino acid ranged from 3.31±0.09 to 13.38±0.63 % on dry weight basis. Most mushrooms exhibited presence of 20 amino acids except *T. rufum* which did not have tyrosine in its fruiting body. Few amino acids as Aspartic acid, Glutamic acid, Asparagine, Glutamine, Glycine,

Table 3. Amino acid composition of wild edible mushrooms (on dry weight basis)

Amino acids (%)	***T. clypeatus***	***T. eurrhizus***	***T. heimii***	***R. nigricans***	***R. brevipes***	***L. fusipes***	***L. tuberregium***	***V. volvacea***	***T. rufum***
Aspartic acid	0.1345	1.5675	1.5843	0.2193	0.3493	0.3453	0.3405	0.1341	0.2193
Glutamic acid	0.2193	1.8945	1.9102	0.3064	0.2193	0.4034	0.1191	0.2013	0.0314
Asparagine	0.3365	0.0893	0.0926	0.1329	0.1031	0.1121	0.3045	0.1143	0.1215
Serine	0.4034	0.0046	0.0059	0.1041	0.2234	0.3042	0.1021	0.1943	0.0093
Glutamine	0.5134	0.9651	0.9752	0.1121	0.1934	0.1191	0.3314	0.0931	0.1131
Glycine	0.6045	0.1645	0.1863	0.3043	0.1054	0.4012	0.2034	0.1354	0.3032
Threonine	0.2135	0.3903	0.4163	0.1102	0.1194	0.1192	0.1131	0.0931	0.1021
Arginine	0.2936	0.0565	0.0587	0.3454	0.0039	0.5042	0.3014	0.1121	0.2193
Alanine	0.3345	0.1093	0.1233	0.1021	0.0931	0.1132	0.1134	0.093	0.3135
Cysteine	0.4032	0.8093	ND	0.1934	0.1935	0.3234	0.2834	0.3155	0.2245
Tyrosine	0.1215	0.1766	0.8253	0.2234	0.2064	0.1045	0.3043	0.1835	Not detected
Histidine	0.3643	0.1813	0.1957	0.1931	0.1015	0.2131	0.2156	0.0215	0.1193
Valine	0.1193	0.1903	0.2143	0.2035	0.2834	0.1131	0.1906	0.1935	0.3042
Methionine	0.0956	0.4934	0.5141	0.0456	0.3091	0.2343	0.1214	0.2164	0.2915
Isoleucine	0.1347	1.5678	0.9784	0.8834	0.1918	0.1031	0.3393	0.1921	0.1364
Phenylalanine	0.2016	0.7733	0.7968	0.1215	0.1145	0.1214	0.1214	0.3343	0.2196
Leucine	0.3193	1.3726	1.3927	0.3132	0.1093	0.2031	0.1634	0.1025	0.3019
Lysine	0.2016	1.7887	1.7996	0.2035	0.1218	0.5436	0.2935	0.4193	0.1121
Proline	0.1375	0.5015	0.5363	0.1131	0.2019	0.0043	0.1021	0.0545	0.3203
Tryptophan	0.0935	0.2895	0.3015	0.2013	0.1132	0.1021	0.0993	0.1125	0.1154
Total ± Std	**5.24±0.14**	**13.38±0.63**	**12.90±0.52**	**4.43±0.17**	**3.35±0.08**	**4.48±0.15**	**4.16±0.09**	**3.31±0.09**	**3.57±0.09**
Essential Amino acids (%)	**24.42**	**49.10**	**47.36**	**42.43**	**37.01**	**31.91**	**32.21**	**46.82**	**40.89**

Threonine, Arginine, Leucine, Lysine and Proline were found abundantly in some mushroom species studied.

Maximum concentration of Lysine, an essential amino acid was in *T. eurrhizus* and *T. heimii* (1.7887 and 1.7996 % w/w) followed by *L. fusipes* and *V. volvacea* (0.5436 and 0.4193 % w/w). Another useful amino acid - Leucine is also found in most of the species studied. *T. heimii* and *T eurrhizus* stand out as amino acid rich species in view of presence of several essential amino acids, i.e., Lysine, Isoleucine, Leucine and Threonine in good amount. Both the mushrooms contained higher amount of Aspartic acid, Glutamic acid, Glutamine, Threonine, Cysteine, Tyrosine, Methionine, Leucine, Phenylalanine, Lysine, Proline and Tryptophan as well. Six essential amino acid Threonine, Methionine, Isoleucine, Phenylalanine, Leucine and Lysine are abundant in these two species. *Russula nigricans* also has good amount of Valine, Isoleucine, in their fruiting body. The presence of cysteine could not be traced out in mushrooms studied where as *L. tuberregium* contained good amount of Proline.

Fatty acids

Table-4 shows the fatty acid profile of wild edible mushrooms analysed in our study. The results of fatty acid composition, included saturated fatty acid and unsaturated fatty acid exhibited presence of three saturated fatty acid palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), one mono-unsaturated oleic acid (C18:1), poly-unsaturated fatty acid like linolenic acid (C18:2), alpha linoleic acid (C18:3) and Moroctic acid (C18:4). Polyunsaturated fatty acid alpha linolenic acid was present in higher amount (0.6503 g/100g) in *T. clypeatus* followed by *T. eurrhizus* (0.2034 g/100g). The presence of alpha linolenic acid was also found in *T. heimii*(0.1983 g/100g) and *Russula nigricans* (0.1931 g/100g). *T. heimii, T. eurrhizus* and *R. nigricans* recorded higher amount of linolenic acid (0.3094, 0.3103, 0.3245 g/100g respectively. Margaric acid was found in almost all mushroom studied except *Tuber rufum similarly.* Moroctic acid is present in six edible mushrooms studied, except in *T. clypeatus, R. brevipes and L. fusipes*. In contrast, *R. nigricans* has higher proportion of linoleic acid (0.32 %). On the other hand, *T. heimii, T. eurrhizus, L. fusipes*and *T. rufum* contained higher amount of saturated fatty acids i.e. stearic acid. Meanwhile *L. tuberregium* showed higher content of saturated palmitic ad (0.55 %) as well as unsaturated linolenic acid (0.51 %). Margaric acid was present in higher amount in *L. tuberregium* and *T. clypeatus* whereas it was absent in *T. eurrhizus*.

Table 4. Fatty acid composition of wild edible mushrooms of Odisha

Fatty acid (%)	***T. clypeatus***	***T. heimii***	***T. eurrhizus***	***R. nigricans***	***R. brevipes***	***L. fusipes***	***L. tuberregium***	***V. volvacea***	***T. rufum***
Palmitic acid (C 16)	0.121	0.157	0.161	0.009	0.083	0.031	0.546	0.031	0.103
Margaric acid (C 17)	0.195	ND	ND	0.021	0.112	0.002	0.203	0.069	nil
Stearic acid (C 18)	0.019	0.305	0.309	0.098	0.109	0.333	0.134	0.013	0.404
Oleic acid (C 18:1)	0.304	0.466	0.487	0.091	0.082	0.403	0.503	0.031	0.295
Linolenic acid (C 18:2)	0.119	0.309	0.310	0.324	0.091	0.134	0.123	0.066	0.256
á-linolenic acid (C 18:3)	0.650	0.198	0.203	0.193	0.124	0.003	0.009	0.032	0.102
Moroctic acid (C18:4)	Nil	Traces	Traces	Traces	Nil	Nil	Traces	Traces	Traces
Total±STDV	1.410± 0.22	1.435± 0.11	1.471± 0.11	0.738± 0.11	0.602± 0.01	0.908± 0.17	1.519± 0.21	0.244± 0.02	1.160± 0.12
Unsaturated fatty acids (%)	75.88	67.83	68.02	82.19	48.33	60	41.72	54.16	56.03

Vitamins

All studied mushroom possess good amount of vitamins. Vitamin A was most abundant in *V. volvacea* (20.4 IU) followed by *R. nigricans* (12.30 IU) and *T. clypeatus* (8.04 IU) which was absent in *L.fusipes* and *L. tuberregium*. Vitamin D was detected in *T. eurrhizus* (13. 44 IU) and *T. heimii* (15.3 IU) only. *T. eurrhizus* and *T. heimii* showed presence of vitamin B_1 and B_2. The amount of vitamin B_6 ranged from 0.113 mg/100g to 2.45 mg/100g the highest proportion being in *T.rufum*. The vitamin B_{12} content was only noticeable in *T. rufum and T. clypeatus*(0.112mcg/100g) where as in other mushrooms it was not traceable. The niacin content was within range of 4 to 14mg/100gm. Folate was detected in *T. eurrhizus* (6.51 mg/100g) and *T. heimii* (7.03 mg/100g) only.

DISCUSSION

Mushrooms are recognized source of plant proteins (Aremu *et al.*, 2009). However there is a wide variation in protein content even within genus. Intra-generic variation is quite distinct as seen in case of *Termitomyces* which ranged from 4.90 to 20.53 mg /g protein. Similarly, *Russula* species had variation from 2.36 to 0.80 mg /g protein and *Lentinus* species had protein content in the range of 15.80 to 11.3 mg /g. In the present investigation the carbohydrates were present in appreciable amount but less than one which was reported by Singdevsachan *et al.* (2012) in *Lentinus* sp.

The wild mushrooms in general have low amount of fat making the mushroom nutritionally ideal diet (Longvah and Deosthale, 1998; Diez and Alvarez, 2001; Agahar-Murugkar and Subbulakshmi, 2005; Barros *et al.*, 2007). The level of protein and fat however may differ for the same species growing in different climatic zone. Johnsy *et al.* (2011) found 30.5 and 37.13 mg/g protein in *V. volvaceae* and *L. tuberregium* compared to our results which are 10.16 mg/g and 15.8 mg/g respectively for the same two species. The difference in the biochemical constituents which agroclimatic effect make it more important to analyze wild edibles from different regions of India. *T. eurrhizus and T. heimii* have not only good range of vitamins and protein but contain low cholesterol (2.41 and 2.44 %) which is even less than crude fat level of soybean (22. 8 - 23.51 %) and pumpkin seed (49.2 - 47.0 %) reported by Salunke *et al.* (1985) and Aisegbu (1989), respectively.

Table 5. Vitamin compositions of wild edible mushrooms

Parameters	*T. clypeatus*	*T. eurrhizus*	*T. heimii*	*R. nigricans*	*R. brevipes*	*L. fusipes*	*L. tuberregium*	*V. volvacea*	*T. rufum*
Vit-A IU	8.040	0.020	0.050	12.300	4.330	0.000	0.000	20.400	4.330
Vit-D IU	0.000	13.440	15.300	0.000	0.000	0.000	0.000	0.000	nil
Vit- B1 mg/100g	3.900	4.090	5.440	ND	ND	ND	ND	ND	ND
Vit-B2 mg/100g	3.500	3.033	4.090	ND	ND	ND	ND	ND	ND
Vit-B6 mg/100g	0.202	1.670	1.890	1.360	0.334	0.346	0.113	2.340	2.450
Vit-B12 mcg/100g	0.112	0.050	0.050	Traces	Nil	Nil	Nil	Traces	0.404
Niacin mg/100g	5.000	4.000	8.000	5.000	5.000	8.000	4.000	14.000	5.000
Folate mg/100g	6.250	6.513	7.033	ND	ND	ND	ND	ND	ND
Vitamin -C gm/100g	0.54±0.05	0.32±0.08	0.75±0.04	0.30±0.06	0.42±0.04	0.30±0.041	0.40±0.09	10.40±0.79	0.69±0.06
Vit E IU	0	ND	ND	0	0	0	0	0	0
Vit K IU	0	ND	ND	0	0	0	0	0	0

Plant fibre is an essential ingredient of good food which is found in varying quantity in vegetables and fruits. Presence of substantial amount of fibre in mushroom fruiting body contributes dietary value and responsible for higher amount of ash in mushroom fungus (Cheung, 1998). Edible mushrooms are reported to be effective in preventing artherosclerosis due to its high fibre constituent. Most mushroom species we studied have good fibre content with the highest amount being in *T. heimii* (37.31 %) and are comparable with legumes, such as pigeon pea and cowpea (Aremu *et al.*, 2006) indicating advantage of such source for human nutrition.

The ash content of the studied mushrooms ranged between 3.63±0.32 to 7.36±0.272 %. The ash content was less in our type materials of *L. tuberregium*, *V. volvacea*and *T. heimii* compared to what has been reported for these species elsewhere. These differences may be attributed to habitat specificity and ecological zones (Bano and Rajarathnam, 1986; Johnsy *et al.*, 2011; Atri *et al.*, 2012).

The mineral content varied significantly at intra generic level of *Russula sp.*, especially Mg, Ca other mineral like P, K, Na were more or less similar in both the species. However, this phenomenon is varied in case of *Termitomyces* where mineral status of individual species differed.

Zn content in different mushrooms ranged between 5.5 – 253 µg/g [4, 55]. Similar variations of concentration in our samples were also observed. Some heavy metals like copper although were detected in species such as *T. heimii* (0.998 mg/100g) and in *T. eurrhizus*(1.33 mcg/100g) the concentration is well within the acceptable health risk level (Kalac and Svoboda, 2000; Ayaz *et al.*, 2011) as up to 10-30 mg/kg dry weight range of copper in food material is considered safe for human.

Many macrofungal species are effective accumulators of selenium (Borovicka and Randa, 2007). We detected selenium only in two species, i.e., *T. eurrhizus* and *T. heimii*. Selenium was not detected in other species that we analyzed. Potassium considered an essential element for human health is also found substantially in mushrooms. Potassium rich diet is considered useful to balance high blood pressure and prevent heart stroke. The wild mushrooms of Odisha consumed by tribal are seen low in cholesterol and have added advantage of having very negligible amount of Na. Appreciable level of phosphorus was also recorded in our samples which was more than the few other edible species analyzed i.e. *Pleurotus* sp. (Ezeibekwe *et al.*, 2009).

A good amount of iron content in *T. eurrhizus* (5975.00 ppm), *R. brevipes* (2373 ppm), *R. nigricans* (2950.33 ppm) and Zn content (68.66 ppm) in *R. brevipes* signifies their mineral accumulator capacity and confirm the findings of Borovicka and Randa (2007) who reported *R. atropurpurea* as a Zn accumulator.

The therapeutic properties of many mushrooms are related to its biochemical constituents of which AA is considered very important (Bernas *et al.*, 2006; Dembitsky *et al.*, 2010). The wild mushroom species consumed of Odisha were seen to be rich in AA compared to other species of edible mushrooms reported by Guzman *et al.*, (1997). Presence of Lysine in *T. eurrhizus*, *T. heimii*, *L. fusipes* and *V. volvacea*make them more nutritious as Lysine is an essential amino acid and very useful in human diet. The result informs that *T. eurrhizus*and *T. heimii* can be a good source of essential amino acid, since in addition to lysine, these species also possess significant amount of leucine and isoleucine (1.3726 and 1.3678 % w/w) .

The quality, quantity and easy availability of free amino acids often determine suitability of food source. Hence, profiling of free amino acid from mushroom is of great importance. As because AA such as Aspartic acid and Glutamic acid contributes to over

alltastes of the food (Guzman *et al.*, 1997).The maximum of total free amino acid found is seen in *T. eurrhizus* followed by *T. heimii* i.e. 13.38 and 12.90 % respectively. Other species showed more or less similar quantity of free amino acids. The presence of essential amino acid was highest in *T. eurrhizus* (49.10 % and 47. 36 %) and *T. rufum* (40.89 %).

Presence of good amount of Aspartic acid, Glutamic acid, Isoleucine, Threonine, Methionine, Cysteine and lysine was reported in *L. tuberregium* cultivated in paddy straw (Manjunathan and Kaviyarasan, 2011). In our samples good amount of proline was present (0.1021 % w/w) compared to other studies (Atri *et al.*, 2012).

Mushrooms in general is reported to be good source of Proline, lysine, arginine, histidine and threonine compared with fruits and Vegetables but poor in Methionine and phenylalanine content compared to egg protein. Another relevant observation we made with reference to level of proline and histidine which are of much higher level than artificially cultured varieties (Dundar *et al.*, 2008). Concentration of aspartic acid and lysine in case of studied samples compared to others highlight the value of the natural resource of the region (Dundar *et al.*, 2008).

This study confirmed presence of 7 fatty acids in wild edible mushroom of Odisha. Two most essential fatty acid i.e. oleic acid and linolenic acid were detected in the analysed species. Presence of unsaturated fatty acid in all three *Termitomyces*sp, *L. fusipes* and *L. tuberregium* is significant from nutritional point of view and indicate importance of the macro fungus for tribal nutrition. In fact it is known that linolenic acid is the precursor of aromatic compound in most fungi and might be contributing to mushroom flavour also (Barros *et al.*, 2007; Barros *et al.*, 2008a). The high content of linolenic acid is one of the important ingredients that makes mushrooms a healthy food (Grangeia *et al.*, 2004; Dembitsky, *et al.*, 2010). Further it has an added advantage having higher proportion of unsaturated fatty acid relative to saturated fatty acid (Longvah and Deosthale, 1998; Diez and Alvarez, 2001).

Vitamins are essential for normal growth and development. Mushrooms are known for its vitamin and reported in several edible mushrooms (Bano and Rajarathnam, 1986; Atri *et al.*, 2012). The specimen sample from Odisha revealed richness of Vitamin B complex in these edible mushrooms.*T. eurrhizus* and *T. heimii* showed good amount of thiamine i.e. 4.09 mg/100g and 5.44 mg/100g, respectively. The results are in agreement with the similar nature of work reported for wild edible mushrooms in term of vitamins (Chang and Buswell, 1996; Baros *et al.*, 2008a; Baros *et al.*, 2008b; Atri *et al.*, 2012). The natural occurrence of vitamins in mushrooms makes them useful source to tap natural nutrient supplements.

Wild edible mushrooms are being collected for consumption as food both by tribals and non tribal villagers. For poor tribal of Odisha it turns out to be a rich source of digestible protein, carbohydrate, fibre fat and vitamin. Among them riboflavin, biotin and thiamine are commonly reported (Crisan and Sands, 1978). Atri *et al.* (2012) reported presence of Vitamin A and Thiamine is present in *T. heimii* but even compared to their result the presently investigated taxa contained higher amount of Vitamin A, B_1 and B_2 in same species (Atri *et al.*, 2012)). The difference in vitamin content could be on account of its habitat which affects secondary metabolism leading to production and synthesis stage of maturity of tissues of collection of vitamins. The biochemical constituents are likely to be influenced by soil, litter, moisture and fungal characteristics to utilize the substratum.

Considerable information on the protein, carbohydrate, amino acid composition and mineral content of several edible mushrooms are reported but their major vitamin content have not been reported except few like *Lentinus* sp. and *Pleurotus* sp. (Bano

and Rajarathnam, 1986; Atri *et al.*, 2012). *Pleurotus* as class of edible mushroom is gaining popularity for many years. It is being cultivated in many parts of the world. As compared to *Pleurotus* sp that contained 92-144 mg/100 g dry weight Ascorbic acid, species of Odisha had ascorbic acid in range of 290-690 mg/100 g in wild mushroom analysed in the present study. Similarly, vit B1 (Thiamine) was recorded in the range of 3.9-5.44 mg/100 g in three species of *Termitomyces* which is quite high compared to the specimens collected from elsewhere (Atri *et al.*, 2012).

The mushrooms analysed from wild sources of Odisha are found to have ideal combination of carbohydrate and proteins with a low fat content. The nutritional quality and unique flavour of these mushrooms need to be therefore exploited by making it a popular food source, for which commercial production protocol is to be developed for each species.

ACKNOWLEDGEMENTS

The financial assistance obtained from Ministry of Environment and Forests, Govt. of India (Project no. 22-24/2010 CS.I) and State plan project, Forest and Environment Dept. of Odisha is gratefully acknowledged by the authors.

Conflict of Interest: Authors declares no conflict of interest.

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