

Compositional and nutritional studies on two wild mushrooms from Western Ghat forests of Karnataka, India

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Abstract

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Introduction

Mushrooms have been a part of the human diet since time immemorial, involving a large number of edible species. In most countries mushrooms are an important delicacy because of the unique flavor and texture though they do not contribute a significant portion of the human diet (Valentao et al., 2005). The rural dwellers are exposed to natural vegetation and they prefer edible higher fungi such as mushrooms, puffballs and morels as luxury food since it has important dietary components (Gbolagade et al., 2006). Though more than 2000 species of mushrooms exist in nature, there is only less than 25 species are widely used as food and only a few have commercialized. Mushrooms are an attractive functional food because of their varied chemical constituents (Elmastas et al., 2007). The traditional use of mushrooms as food and medicine in Asian countries are also known (Manzi et al., 1999; Sanmee et al., 2003).

Mushrooms are healthy foods rich with proteins, vitamins, minerals, fibers, trace elements and poor in calorie and cholesterol (Caglarlrmak *et al.*, 2002; Barros *et al.*, 2008). Mushrooms are comparable to meat, egg, and milk because it contains amino acid composition similar to that of animal proteins. Wild

The chemical composition and nutritional value of two wild mushroom species namely, *Amanita hemibapha* (Berk.&Br.) Sacc. and *Trogia cantharelloides* (Mont.) Pat. was determined. Nutritional evaluation includes proximate, amino acid, fatty acid and mineral analysis. The macronutrient profile in general revealed that the wild mushrooms were rich source of carbohydrates and protein, and had low fat content. On the basis of proximate analysis, it can be calculated that an edible portion of 100 g of these mushrooms provides, on an average, 420.083 kcal (1758.802 kJ). Among the total amino acids lysine, leucine, threonine and isoleucine were found as major essential amino acids in both the species. The fatty acid analysis by Gas Chromatography could identify and quantify about 17 and 18 fatty acids in *A. hemibapha* and *T. cantharelloides*, respectively. Linoleic acid (34.56±0.002 g) per 100 g lipid was in *A. hemibapha*. Phosphorous and potassium was the most abundant minerals followed by magnesium in both the species. This study contributes to the documentation of the nutritional composition of *A. hemibapha* and *T. cantharelloides*.

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mushrooms serve as rich source of protein and lower amount of fat compared to commercial mushrooms (Barros *et al.*, 2008) .Wild mushrooms are thus nutritionally rich (Breene, 1990; Manzi *et al.*, 1999) and its consumption is increasing in the developed world (Thimmel and Kluthe, 1998). Western Ghats region is one of the four globally recognized biodiversity hot spots spread along the West Coast of India, which harbor about 750 species of mushrooms and hosts several non-estimated biodiversity and is a treasure of unidentified mushroom species with enormous applications including edibility, which have to be exploited to strengthen the food security of a country (Lakhanpal, 1994).

In the present work, the chemical composition of the mentioned mushroom species were evaluated [*Amanita hemibapha* (Berk. and Br.) Sacc. and *Trogia cantharelloides* (Mont.) Pat.]. In this study the proximate composition, amino acid, fatty acid and mineral composition of two mushroom species were analyzed to evaluate the nutritional value. Regarding their nutritional value no data is available till the date. Therefore it is important to analyze the composition of wild mushrooms to know its nutritional potentiality.

Materials and Methods

Macrofungi

Two edible wild mushroom, Amanita hemibapha (Berk. & Br.) Sacc. and Trogia cantharelloides (Mont.) Pat. were collected from Hebri region of the Western Ghat forests of Karnataka (13°28'N, 74°59'E). The mushrooms were transferred to laboratory immediately after collection and the fruiting bodies were cleaned thoroughly (without washing) from soil and forest debris with soft tissue paper and cut into small pieces with plastic knife. Later the samples were dried at 60°C in hot air oven and were ground to fine powder and stored at -20°C for further analysis. Taxonomic identification was done based on visual inspection on morphological features of specimens and microscopic observation of their spores combined with the use of identification keys from reference book (Mohanan, 2011).

Proximate analysis

The proximate compositions of the two species of mushrooms, including moisture, ash, carbohydrate, crude fat, crude fiber and crude protein, were determined in triplicate, according to the methods in Association of Official Analytical Chemists (AOAC, 1995). The dried mushroom samples were further heated at 105°C overnight until constant weight obtained for moisture content determination. The samples were incinerated at 550°C for 24 h and reweighed to determine ash content. Micro-Kjeldahl method was employed for the crude protein content (N \times 4.38). The crude fat was determined by extracting a known weight of sample in Soxhlet apparatus using petroleum ether as a solvent. Total carbohydrates calculated by the difference. According to the following equation total energy was calculated: total energy $(kJ) = 17 \times (g \text{ crude protein} + g \text{ total})$ carbohydrate) + $37 \times (g \text{ crude fat})$.

Amino acid analysis

Amino acid analysis was performed by High Performance Liquid Chromatography (HPLC) (Fierabracci *et al.*, 1991). 0.1 g of dried mushroom powder was hydrolyzed with 10 ml of 6N HCl in evacuated sealed tubes for 24 hrs at 110°C. Later, the hydrolyzed samples were allowed to cool. The sample was filtered using Whatman filter paper; No.42 into a round bottom flask and evaporated till the acid content is completely removed. Finally, the volume was made up to 5 ml with 0.05N HCL and derivatized to phenyl thiocarbomyl amino acid before injected to HPLC.

Fatty acid analysis

Soxhlet method was used to extract fatty acids from mushroom powder (Xiao et al., 2012). The extracted fatty acids were esterified and then FAMEs were diluted (40 ml FAME sample + 960 ml n-hexane) in the sample vial. 1 µL of methyl esterified sample was injected to the chromatograph (GC-2010, Shimadzu, Kyoto, Japan), by an auto injector (AOC-20i, Shimadzu) and capillary column (BPX 70, SGE Analytical Science, Austin, TX). Each elutant was detected by the Flame Ionization Detector (Shimadzu). The conditions set for the analysis were followed as per protocol of Nareshkumar (2007). The split injection mode was used with a split ratio of 1:50 (conditions maintained were as follows: terminal temperature was 225°C; nitrogen and air were carrier gases; pressure was set to 114.9 k Pa; total flow was maintained at 68.9 ml/min; and column initial temperature was 100°C with temperature increase rate of 5°C/min).

Minerals

The powdered mushroom samples were digested in a tri-acid mixture of nitric acid, sulfuric acid and perchloric acid (10:0.5:2, v/v).The digested samples were then subjected for Atomic Absorption Spectroscopy (AAS) analysis for microminerals (Fe, Cu, Zn, Se, As) and macrominerals (Na, K, Mg, Ca) (AOAC, 2006). Phosphorous present in the form of orthophosphate in the samples were determined by Vanadomolybdo-Phosphoric acid method (APHA, 1995).

Statistical analysis

The results are expressed in mean \pm S.D. in triplicate. The differences in proximate composition, amino acids, fatty acids and minerals were analyzed by t-test.

Results and Discussion

In Table 1 the results of proximate content and energetic values of the two wild edible mushrooms studied were reported. The moisture content was $82.34\pm0.20\%$ and $96.07\pm0.03\%$ in *A. hemibapha* and *T. cantharelloides* respectively confirming the high moisture content of the mushroom. The result of *T. cantharelloides* was in the 85-95% range which is a normal percent for fresh mushrooms. The results have clearly shown that the moisture contents of the mushrooms analyzed are high, indicating that mushrooms are highly perishable because microbial growth and enzyme activities are more with high moisture content (Breene, 1990). Other than

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Amino acid	Amanita hemibapha	Trogia cantharelloides	Whole egg protein ^b	
		C 0 0 1 0 0 0 **		
Aspartic acid	5.04 ± 0.12	6.98±0.09	9.6	
Glutamic acid	8.75±0.002	12.88 ± 0.02	12.7	
Serine	8.43±0.04	$8.61 \pm 0.05^{***}$	7.6	
Glycine	11.33 ± 0.05	9.10±0.03 ****	3.3	
Histidine	2.70 ± 0.01	2.60±0.01	2.4	
Arginine	6.30±0.02	4.85±0.02***	6.1	
Threonine	4.50±0.25	4.85±0.05	5.1	
Alanine	13.56 ± 0.04	$7.80\pm0.01^{***}$	5.9	
Proline	6.26±0.06	6.25±0.10	4.2	
Tyrosine	2.53 ± 0.001	2.48 ± 0.10	4.2	
Valine	6.02 ± 0.001	$5.65 \pm 0.20^{*}$	6.9	
Methionine	1.48 ± 0.07	$1.23\pm0.03^{**}$	3.4	
Cystein	0.14 ± 0.01	$0.41 \pm 0.04^{**}$	5.9	
Isoleucine	4.23±0.1	4.53±0.06***	6.3	
Leucine	5.62 ± 0.02	6.61±0.08***	8.8	
Phenylalanine	2.10 ± 0.08	3.97±0.04***	5.7	
Lysine	6.76±0.03	6.90±0.20	7	
Tryptophan	ND	ND	ND	

Table 2. Amino acid (g/100 g protein) profile of *Amanita hemibapha* and *Trogia* cantharelloides (n=3, mean \pm SD)a

^aAsterisk across the columns between Amanita hemibapha and Trogia cantharelloides samples denotes significant difference (t-test: *, p < 0.05; **, p < 0.01; ***, p < 0.001)

ND – Not detectable

^b Whole egg protein (FAO, 1968)

Table 1. Moisture content (g/100 g of fresh weight), macronutrients (g/100 g of dry weight) and total energy (kJ/100 g of dry weight) in the two wild edible

Components	Species		
	Amanita hemibapha	Trogia cantharelloides	
Moisture	82.34±0.205	96.07±0.030***	
Ash	0.982±0.076	1.53±0.025**	
Dietary fiber	11.78±0.116	11.1±0.854	
Crude fat	5.6±0.1	2.3±0.1***	
Crude protein	10.53±0.231	9.46±0.305*	
Total carbohydrates	82.89±0.203	86.705±0.373**	
Total energy	1797.58±4.99	1720.024±1.62***	

^aAsterisk across the columns between *Amanita hemibapha* and *Trogia cantharelloides* samples denotes significant difference (t-test: *, p < 0.05; **, p < 0.01; ***, p < 0.001)

mushroom species the factors like environmental temperature, relative humidity during growth and relative amount of metabolic water that may be produced or utilized during storage will also affect moisture content (Crisan and Sands, 1978). In both the species the dominant compounds were carbohydrates, proteins and fibers. Lipid content ranged from $2.3\pm0.1\%$ (T. cantharelloides) to $5.6\pm0.1\%$ (A. hemibapha), the fat content is less compared to other common mushrooms. Carbohydrate was found to be the most abundant macronutrient here, glucans, mono- and disaccharides, sugar alcohol, glycogen and chitin contributes to mushroom carbohydrates (Kurztman, 1997). Cholesterol related ailments may be reduced by intake of fiber rich diet and the results here showed appreciable quantity of fibers which are known as anti-tumorigenic and hypocholestrolemic agents (Kadiri and Fasidi, 1990).

The amino acid composition of the A. hemibapha and T. cantharelloides are given in Table 2. Among the total amino acids lysine, leucine, threonine and isoleucine were found as major essential amino acids both in A. hemibapha and T. cantharelloides. Alanine (13.56±0.04 g), glycine (11.33±0.05 g), glutamic acid $(8.75\pm0.002 \text{ g})$ and serine $(8.43\pm0.04 \text{ g})$ g) per 100 g protein in A. hemibapha and glutamic acid (12.88±0.02 g), glycine (9.10±0.03 g), serine (8.61±0.05 g) and aspartic acid (6.98±0.09 g) per 100 g protein in T. cantharelloides contributed as major non essential amino acids. The presence of significant amount of glutamic acid in both the mushrooms could be relevant since it is involved in the primary umami taste and it is also a known flavor potentiator (Yamaguchi et al., 1971; Yojiro and Morely, 1987). In both the species cystine was found in least concentration (0.14±0.01 g in A. hemibapha and 0.41±0.04 g in T. cantharelloides). The amino acids, serine, glycine, histidine, alanine and proline, exceeded those of whole egg protein. The results clearly show that the mushroom species studied here are rich sources of essential amino acids as it contains all the essential amino acids in adequate quantity except tryptophan.

The results for fatty acid composition, total saturated fatty acids and polyunsaturated fatty acids (PUFA) of studied species are shown in Table 3. There are about 18 fatty acids detected in *T. cantharelloides* and about 17 fatty acids in *A. hemibapha*. The most abundant fatty acid was linoleic acid (34.56 ± 0.002 g) followed by oleic acid (18.53 ± 0.003 g), palmitic acid (10.23 ± 0.002 g) and

Fatty acid	Amanita hemibapha	Trogia cantharelloides
Saturated fatty acid		
Capric acid(C10:0)	ND	0.179±0.002
Lauric acid (C12:0)	0.098±0.003	0.154±0.003**
Myristic acid (C14:0)	0.184±0.000	0.254±0.001***
Penta decanoic a cid (15:0)	0.108±0.001	0.423±0.003***
Palmitic acid (C16:0)	10.626±0.020	10.233±0.002***
Heptadecanoic acid (C17:0)	ND	0.163±0.006
Stearic acid(C18:0)	15.830±0.001	2.683±0.001***
Heneicosanoic acid (C21:0)	0.124±0.007	ND
Behenic acid (C22:0)	ND	0.175±0.000
Tricosanoic acid (C23:0)	0.303±0.002	0.2±0.010**
Lignoceric acid (C24:0)	0.475±0.005	1.735±0.003***
Unsaturated fatty acid	0.10.00.000	200
Myristoleic acid	0.1382±0.006	ND
Palmitoleic acid (C16:1)	0.364±0.005	0.229±0.002***
Oleic acid (C18:1)	38.818±0.005	18.529±0.003***
Linoleic acid (C18:2)	18.641±0.016	34.556±0.002***
Linolenic acid (C18:3) Linolelaidic acid	ND 0.32±0.001	0.10±0.002
		1.24±0.007***
cis-11,14-Eicosadienoic acid (C20:1)	1.339±0.001	ND
cis-4,7,10,13,16,19-	ND	9.75±0.000
Docosahexaenoic acid (22:6)		
cis-5,8,11,14,17-	1.34 ± 0.001	ND
	1.0 1-0.001	1410
Eicosapentaenoic acid	1.51-0.001	ND
Eicosapentaenoic acid Total saturated fatty acids	27.75±0.039	16.19±0.031***

Table 3. Fatty acid methyl esters (g/100 g lipid) of *Amanita hemibapha* and *Trogia* cantharelloides (n=3, mean ± SD)^a

^a Asterisk across the columns between Amanita hemibapha and Trogia

cantharelloides samples denotes significant difference (t-test: *, p < 0.05; **, p < 0.01;

****, p < 0.001)

ND - Not detectable

^bRatio of polyunsaturated/saturated fatty acids

docosahexaenoic acid $(9.75\pm0.00 \text{ g})$ and the least was linolenic acid $(0.10\pm0.002 \text{ g})$ in T. cantharelloides. Oleic acid $(38.82\pm0.005 \text{ g})$ was the major fatty acid followed by linoleic acid (18.64±0.016 g), stearic acid (15.83±0.001 g) and palmitic acid (10.63±0.020 g) and the least was lauric acid $(0.098\pm0.003 \text{ g})$ in A. hemibapha. Palmitic, oleic and linoleic acids accounted for almost whole of the fatty acids same as the results of Schizophyllum commune and Lentinus edodes (Longvah and Deosthale, 1998). The majority of the studies on wild and cultivated edible mushrooms revealed that the main fatty acid found is linoleic, oleic and palmitic acids (Barros et al., 2008; Jing et al., 2008). The polyunsaturated to saturated fatty acid ratio was high for T. cantharelloides (3.97) compared to A. hemibapha (2.19). Both the mushrooms studied here had ratios above 0.45 indicating that these are healthy foods because the ratios greater than or equal to 0.45 have hypocholesterolemic effect (Chang and Huang, 1998). Linoleic, oleic and palmitic acids are the predominant fatty acids here as reported in other Basidiomycetes (Yilmaz et al., 2006; Pedneault et al., 2006). Palmitic and stearic acids are the best saturated fatty acids among natural fat for human

nutrition (Hayes, 2002). The high amount of linoleic acid in these mushrooms supports that mushrooms as a health food as unsaturated fatty acids are very important in human diet (Lawrence, 2010).

The mineral profile is given in Table 4. The important physiological functions like metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance are maintained by dietary intake of minerals. Phosphorous was the most abundant mineral found here in both the species. This was followed by potassium, calcium and magnesium. This result was in agreement with the results obtained for Clavatia gigantea, Cantharellus cibarius, Rusulla integra, Lactarius quieticolor, Gomphus floccosus, Ramaria brevispora and Clavulina cineria where phosphorous was the abundant mineral and selenium was in low concentrations (Agrahar-Murugkar and Subbulakshmi, 2005). Trace elements such as iron, copper, zinc were found in adequate quantity in these mushrooms. The great amount of potassium and low content of sodium suggests the use of mushroom in an anti-hypertensive diet as potassium from fruit and vegetables can lower blood pressure (Lakhanpal,

Table 4. Mineral (mg/100 g) profile of *Amanita* hemibapha and *Trogia cantharelloides* $(n=3, mean \pm SD)^a$

Mineral	Amanita hemibapha	Trogia cantharelloides
Fe	5.910±0.08	0.387±0.007***
Cu	0.050±0.01	0.034±0.011
Р	514.58±0.42	260.42±0.401***
Mg	7.897±0.019	$1.148\pm0.004^{***}$
Ca	8.420±0.04	1.274±0.005
Na	1.148 ± 0.01	$0.782 \pm 0.012^{***}$
K	14.640 ± 0.18	12.560±0.060 ^{**}
Se	0.113±0.01	$0.092 \pm 0.020^{*}$
Zn	0.374 ± 0.004	$0.002 \pm 0.000^{***}$
As	0.335 ± 0.005	$0.623 \pm 0.002^{***}$

^aAsterisk across the columns between *Amanita hemibapha* and *Trogia cantharelloides* samples denote significant difference (t-test: *, p < 0.05; **, p < 0.01; ***, p < 0.001)

1994).

The maintenance of acid-base balance, the osmotic regulation of fluid and oxygen transport in the body are the major functions of macronutrient minerals such as sodium, magnesium, copper, cobalt, chromium and molybdenum (McDowell, 2003), whereas micronutrient minerals (Zn, Se, Mo, Fe, Cu, V, Cr, and Co) are important in catalytic process of wide range of enzyme activities associated with the metabolic, endocrine and immune systems (Keen *et al.*, 2004). Therefore, the lack of mineral intake through diet, intestinal absorption and cellular uptake can cause for diseases or infections through their negative impact on immune function (Kiremedijian-Schumacher *et al.*, 1994; Ekiz *et al.*, 2005).

Conclusions

The data obtained in this work add to the scarce information on Amanita hemibapha and Trogia cantharelloides. The nutrient profile of Trogia cantharelloides is described for the first time. The chemical composition and energy values of the wild mushrooms clearly indicate that they provide key nutrients such as carbohydrates, protein and unsaturated fatty acids. Information regarding antinutritional and toxic factors in wild mushrooms is also required for its wide acceptance.

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