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Nutritional perspectives of an ectomycorrhizal edible mushroom *Amanita* of the southwestern India

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Abstract

The occurrence of ectomycorrhizal *Amanita* sp. is common in scrub jungles of southwest India and tender sporocarps serve as ethnic nutritional source for local dwellers during southwest monsoon season. Evaluation of the nutritional constituents of uncooked and cooked tender sporocarps revealed significantly higher quantity of total lipids and calorific value in uncooked than cooked samples, while it was opposite for the crude protein. There was no significant change in crude fibre and carbohydrates between uncooked and cooked samples. Uncooked as well as cooked samples were rich in potassium followed by iron. The Na-K ratio in uncooked as well as cooked samples (<1) was favourable, while the Ca-P ratio (<1) was not favourable. In cooked samples, most of the essential amino acids (histidine, isoleucine, methionine, cystine, phenylalanine, tyrosine, threonine and valine) were significantly increased. The *in vitro* protein digestibility was significantly higher in uncooked than cooked samples. The protein digestibility corrected to amino acid score was moderate to high (uncooked, 58-104; cooked, 54-91). The protein efficiency ratios in uncooked and cooked samples (>2) depicts the high quality of protein. Among the fatty acid methyl esters, oleic acid in uncooked samples, while palmitic and stearic acids in cooked samples were significantly higher. The tender sporocarps of *Amanita* sp. in scrub jungles of southwestern India provide valuable nutrients to the local dwellers during monsoon period.

Key words – Amino acids – fatty acids – minerals – protein bioavailability – proximal qualities

Introduction

Similar to the plants and animals, fungi have an independent evolutionary line capable to meet human requirements like nutrition, medicine and industrial applications. Hypogeous or epigeous macrofungi possess distinct characteristic macroscopic fruit bodies (Chang & Miles 1989). They are the centre of attraction worldwide as they constitute important live material for production of enzymes, metabolites, cosmetics and nanomaterials (Manzi & Pizzoferrato 2000, Wu et al. 2004, Hyde et al. 2010, Vikineswary & Chang 2013, Arun et al. 2014, Taofiq et al. 2016). One of the interesting features of wild macrofungi is that their benefits (nutritional and therapeutic value) are still recognized based on traditional knowledge of tribes or native people of a specific geographic region. Asian countries are known for utilization of macrofungi for human nutrition and therapy based on ethnic knowledge (Aly et al. 2011, Xu et al. 2011, De Silva et al. 2013). The Western Ghats of India being one of major hotspots of biodiversity, known for a variety of macrofungi grow in a wide range of forest ecosystems at different altitudinal ranges (Mohanan

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2011, Farook et al. 2013, Senthilarasu 2014, Pavithra et al. 2015, Senthilarasu & Kumaresan 2016). Systematic inventories and discussion with tribals and native people resulted in recognizing many edible mushrooms in the Western Ghats and west coast of India (Ghate et al. 2014, Senthilarasu 2014, Karun & Sridhar 2014, 2016, Pavithra et al. 2015). *Amanita* sp., *Astraeus* spp., *Auricularia* spp., *Lentinus* spp., *Russula* spp. and *Termitomyces* spp. are some of the commonly traditionally consumed macrofungi in the Western Ghats and west coast of India (Senthilarasu 2014, Karun & Sridhar 2014, 2016, Pavithra et al. 2015).

The range of *Amanita* spp. worldwide represented from 900-1000 species (Tulloss 2005). This genus consists of over 500 ectomycorrhizal species associated with diverse tree species (e.g. *Abies, Cedrus, Picea* and *Pinus)* (Itoo et al. 2016). Although several members of Amanitaceae are poisonous, many are edible (e.g. *Amanita caesarea, A. chepangiana, A. citrina, A. crocea, A. flammeola, A. franchetii, A. fulva, A. hemibapha, A. jacksonii, A. manginiana, A. loosii, A. pseudoporphyria, A. princeps, A. rubescens, A. tuza, A. sinensis, A. vaginata* and *A. zambiana* (Pegler & Piearce 1980, Bhatt & Lakhanpal 1988, León-Guzmán et al. 1997, Ouzouni 2007, Pérez-Moreno et al. 2008, Sanmee et al. 2008, Semwal et al. 2014, Tripathy et al. 2014). Recent inventories in the lateritic soils of scrub jungles in southwestern India revealed occurrence of *Amanita* sp. which is traditionally considered edible in tender stage (Karun & Sridhar 2014). This mushroom has association with many tree species in scrub jungles (e.g. *Acacia auriculiformis, Anacardium occidentale, Hopea ponga* and *Terminalia paniculata*). It is a traditional practice to collect tender sporocarps in different shapes (spherical, oval, dumble and partly ruptured volva) for consumption. The fruit bodies show up for a short period during southwest monsoon season (June-July). Being edible in young stage, such fruit bodies of *Amanita* sp. were collected from the scrub jungles in lateritic belt of southwestern India and evaluated its nutritional potential in uncooked and cooked form.

Materials & Methods

Mushroom

Edible stages of *Amanita* sp. (young sporocarp stages) were collected from the lateritic soils of the southwestern India (Konaje Village, Dakshina Kannada, Mangalore, India: 12°48'N, 74°55'E; 115 m asl) with support of local dwellers who regularly consume during monsoon season (June-August). Its fruit bodies are very common underneath the tree species of *Acacia auriculiformis, Anacardium occidentale, Hopea ponga* and *Terminalia paniculata*. Based on macro- and micro-morphological features, although the *Amanita* sp. roughly matches with *Amanita marmorata* reported from Hawaii (Miller et al. 1996), several glaring differences support to consider it as a new species. The tender sporocarps collected and consumed by the villagers include spherical, oval, dumble shapes and just partially ruptured volva stage (Fig. 1a-k). Sampling was carried out in five locations with about 50 m apart in lateritic scrub jungles. The young stages of mushroom in each sample were separately rinsed in distilled water to eliminate soil, roots and other debris. They were wiped with clean cloth to eliminate moisture on the surface. Each replicate was divided into two groups, the first group was oven dried at 50-55°C, while the second group was separately cooked in a household pressure-cooker with distilled water (1:1 v/v) followed by oven drying. The dried samples were milled in Wiley Mill (mesh #30) and powder was refrigerated in air-tight containers for analysis.

Proximal analysis

Moisture. Moisture content of uncooked and cooked mushroom powder of *Amanita* sp. was assessed gravimetrically (AOAC 1995). Replicate flour samples were dried at 80°C for 24 hr and difference between initial and final weight were considered to estimate moisture content in per cent to express proximate composition on dry weight basis.

$$\text{Moisture (\%)} = \left(\frac{I-F}{W}\right) 100$$

(where I, weight of sample before drying in g; F, weight of sample after drying in g; W, weight of mushroom flour taken in g).

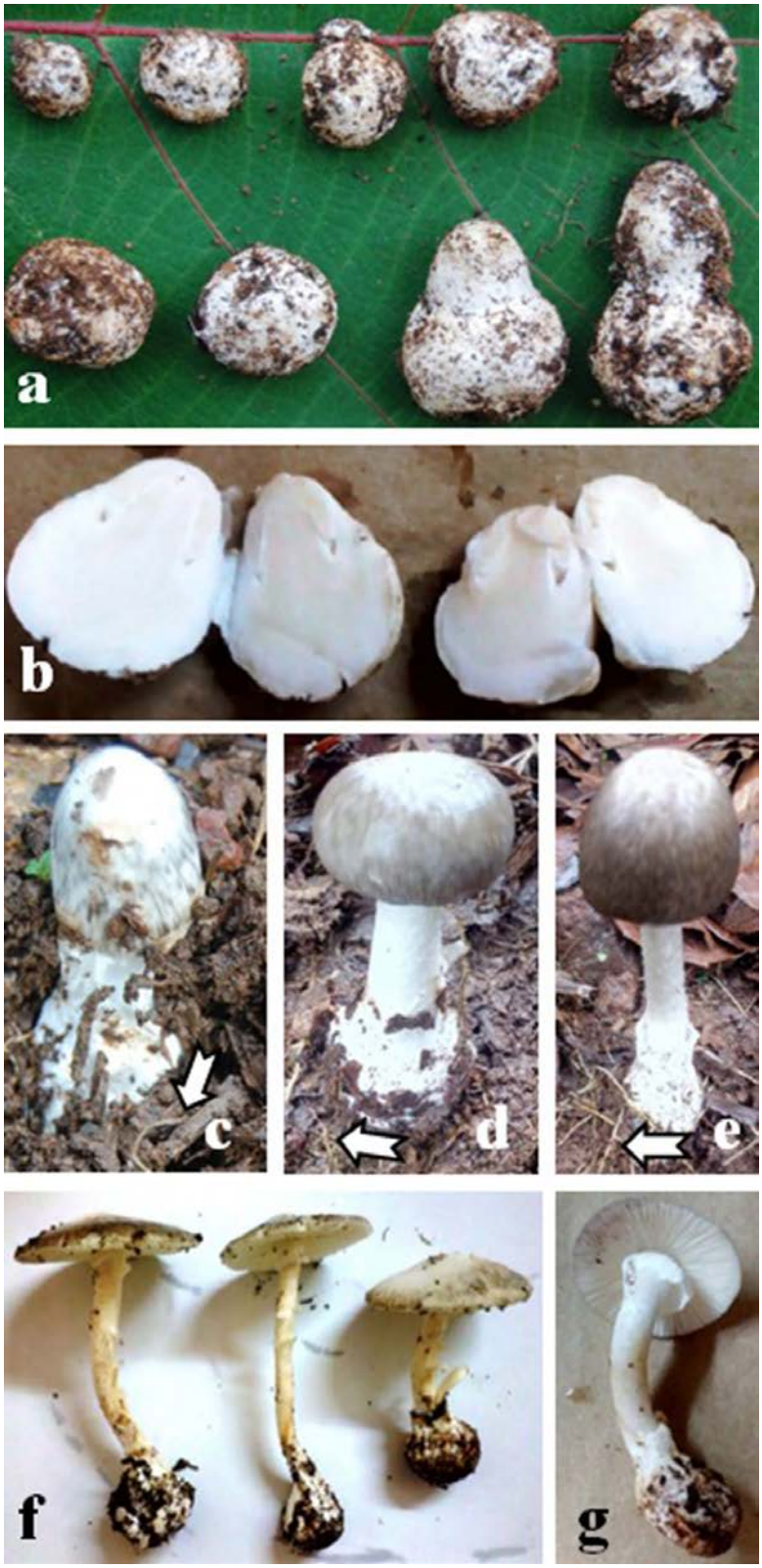

Fig. 1 – Various stages of immature sporocarps a-b maturing sporocarps. c-e and mature fruit bodies. f-g of *Amanita* sp.: spherical, beak-like protuberance, extended protrusion and dumble shaped tender sporocarps a cut-open tender sporocarps. b extended stipe prior to opening of pileus. c-e (note roots surrounding volva, arrows); mature fruit bodies. f gills, partial veil with intact volva of a mature fruit body (g).

Crude protein. The crude protein content was evaluated by micro-Kjeldahl method (Humphries 1956). Mushroom flour (100 mg) was extracted with pinch of catalytic mixture (copper sulphate, selenium, potassium sulphate: 1:1:20 w/w), concentrated sulphuric acid (10 ml). The mixture was digested in Kjeldahl flasks until it turns colourless and the volume was made up with distilled water (100 ml). The digested sample (10 ml) was transferred to Kjeldahl apparatus, sodium hydroxide (40%, 10 ml) was added and distilled until accumulation of 25 ml in receiver flask containing boric acid (2%, 10 ml) and mixed indicator (0.2 % of methyl red and methylene blue in ethanol, 2:1 v/v). After cooling to room temperature, the solution was titrated against hydrochloric acid (0.01N) till the colour changes from green to pink and nitrogen content was calculated to determine crude protein content.

$$\text{Nitrogen (\%)} = \left(\frac{\text{A} \times \text{N} \times 0.0014}{\text{W}}\right) 100$$

(where A, volume of 0.01N HCl titrated minus volume of blank; N, normality of HCl; 0.0014, g nitrogen in 0.1N HCl; W, weight of sample in g).

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Total lipids. The total lipid was determined based on the method of AOAC (1995). Mushroom flour (~1 g) was extracted with petroleum ether (6 hr) in Soxhlet apparatus. The solvent was evaporated to dryness. The initial and final weight of the sample was recorded to calculate the percentage of total lipid.

$$\text{Total lipids (\%)} = \left(\frac{\text{F–I}}{\text{W}}\right) 100$$

(where I, weight of empty flask in g; F, weight of flask with lipid in g; W, weight of mushroom flour taken in g).

Crude fibre. The crude fibre content was determined gravimetrically according to AOAC (1995). Defatted mushroom sample (500 mg) was treated with sulphuric acid (0.025N, 200 ml) and boiled (30 min). On cooling the contents were filtered, the residue was washed repeatedly in boiling distilled water to eliminate acid traces. The residue was boiled in sodium hydroxide (0.313N) (30 min). The contents were filtered and washed repeatedly in boiling water to remove traces of alkali. The residue was transferred to pre-weighed crucible, heated in muffle furnace (550°C, 3 hr) and the final weight was recorded on cooling.

$$\text{Crude fibre (\%)} = \left(\frac{\text{F} - \text{I}}{\text{W}}\right) 100$$

(where I, weight of empty crucible in g; F, weight of crucible with fibre in g; W, weight of mushroom flour taken in g)

Ash. The percentage of ash was determined based on AOAC (1995). The homogenised mushroom flour (~1 g) was taken in pre-weighed porcelain crucible and dried in the oven at 100°C for 6–8 hr. The crucible was then transferred to furnace (550°C, 8 hr) until attaining constant weight to calculate the ash content.

$$\text{Ash (\%)} = \left(\frac{\text{F} - \text{I}}{\text{W}}\right) 100$$

(where I, weight of crucible in g; F, weight of crucible with ash in g; W, weight of mushroom flour taken in g)

Carbohydrates. To evaluate carbohydrate content, the phenol sulphuric acid method proposed by Dubois et al. (1956) was followed. Mushroom sample (100 mg) was treated with hydrochloric

acid (2.5N, 5 ml) and heated in boiling water bath (3 hr). The reaction was neutralized by the addition of sodium carbonate until the effervescence ceases and the volume was made up to 100 ml with distilled water. Sample (0.2 ml) was further diluted with distilled water (0.8 ml), phenol (5%, 1 ml) followed by sulphuric acid (96%, 5 ml) was added and kept in water bath (30°C, 20 min). Control was prepared based on the following method without addition of sample. The absorbance was measured (490 nm). The D-glucose served as standard and the mean value was expressed in gram of carbohydrate in 100 gram of mushroom sample.

Calorific value. The calorific value of the mushroom powder was calculated according to the formula proposed by Ekanayake et al. (1999).

$$\text{Gross energy (kJ/100 g)} = (\text{Crude protein} + 16.7) \times (\text{Total lipids} + 37.7) \times (\text{Carbohydrates} \times 16.7)$$

Mineral analysis

The scanning electron microscope-energy dispersive X-ray spectrometer (SEM-EDX) was employed to evaluate minerals (Lui et al. 2015). Homogenised mushroom flour (particle size, ~40 µm) was dusted on the brass stub with the carbon tape. The mounted powder samples were coated with gold by sputter coat (20 mA, 10 min). The samples were analysed by SEM (Carl Zeiss Sigma, Germany) and EDX (Oxford instruments, Germany) at 3.5 mm working distance. The beam energy used was 15KeV and maps of 98X pixel were obtained in the selected area. The generated mineral maps were assessed for distribution of elemental concentration. The ratios of Na-K and Ca-P were calculated.

Amino acid analysis

The amino acids content in the mushroom flour was assessed based on Hofmann et al. (1997, 2003).

Hydrolysis. Known quantity of mushroom sample was treated with hydrochloric acid (6N, 15 ml, 145°C, 4 hr). Alkaline hydrolysis was followed for tryptophan as they are stable in basic condition and for sulphur amino acids oxidized samples were used. The contents were evaporated to dryness to remove hydrochloric acid on a rotary evaporator (Büchi Laboratoriumstechnik AGRE121; Switzerland) connected to diaphragm vacuum pump (MC2C; Vacuubrand GmbH, Germany).

Derivatization. For derivatization, the hydrolysate was treated with trans-4-(aminomethyl)-cyclohexanecarboxylic acid (purity, 97%; Sigma Aldrich) as internal standard.

Standards. The weighed standards in reaction vial were treated with dichloromethane and dried to remove moisture by flushing inert helium with passive heating in an oil bath (60°C). Acidified isopropanol (12 ml) (acetyl chloride + 2-propanol) (1:4 v/v) was added following by heating (100°C, 1 hr). The contents were evaporated to dryness in the oil bath (60°C) by gently flushing helium. The dry residue was treated with dichloromethane and evaporated; this process was repeated to remove traces of water and propanol. The residues were treated with trifluroacetic anhydride (200 ml) for overnight at room temperature. The fraction of the solution was injected in gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS/MS).

Analytical parameters. Isotope with GC-C-IRMS/MS (GC: Hewlett-Packard 58590 series II, Germany; combustion series II-interface, IRMS MAT 252, Finnigan MAT, Germany; MS: GCQ, Finnigan MAT, Germany) was carried out. The capillary column of dimension 50 m × 0.32 mm i.d. × 0.5 µm BPX5 (SGE) was connected to gas chromatography. The flow of carrier gas was 1.5 ml/min with the head pressure 13 psi. The details of temperature programme are given in Table 1.

Essential amino acids. The ratio of total essential amino acids (TEAA) to total amino acids (TAA) was calculated.

$$\text{TEEA-TAA ratio} = \left(\frac{\text{TEAA}}{\text{TAA}}\right)$$

Table 1 Temperature programme for GC-C-IRMS/MS.

Time (min)	Temperature (°C)	Temperature/min
1	50	start
10	50-100	10°C/min
10	100-175	3°C/min
10	175-250	3°C/min
10	250	stop

The essential amino acid score (EAAS) was calculated by dividing individual EAA content by FAO-WHO (1991) EAA reference pattern.

$$\text{EAAS} = \left(\frac{\text{Amino acid content in the test protein mg/g}}{\text{FAO} - \text{WHO EAA reference pattern mg/g}}\right)$$

Protein bioavailability

Digestibility. The *in vitro* protein digestibility was determined based on multi-enzyme technique (Akeson & Stahmann 1964). Protein digest were prepared by treating 100 mg of mushroom flour with 2.5 ml of 1.5 mg pepsin (3165 units/mg protein, Sigma Aldrich) in 0.1N hydrochloric acid and was neutralized by the addition of 2.5 ml of 1N sodium hydroxide. The reaction mixture was treated with enzyme solution i.e. trypsin (16100 units/mg protein, Sigma Aldrich) and chymotrypsin (76 units/mg protein, Sigma Aldrich) (2 mg each in 2.5 ml of 0.1M phosphate buffer, pH 8), incubated at 37°C for 24 hr. The reaction was halted by the addition of 0.7 ml trichloroacetic acid (100 %). Enzyme blank was prepared as described above but without addition of sample. The contents were centrifuged and supernatant was recovered. The residue was repeatedly washed with 10% Trichloroacetic acid and the supernatant was pooled. The supernatant was treated with twice the volume of diethyl ether and ether layer was gently removed by aspiration. The aqueous layer was maintained in water bath to eliminate the traces of ether. After cooling, the volume was made to 25 ml by distilled water. The nitrogen content in the sample was estimated by micro-Kjeldahl method (Humphries 1956). IVPD was calculated as follows:

$$\text{IVPD (\%)} = \left(\frac{\text{Protein in digest}}{\text{protein in defatted flour}}\right) 100$$

PDCAAS. Protein digestibility-corrected amino acid score (PDCAAS) has been calculated based on FAO-WHO (2007) for the measurement of the protein value in the human nutrition.

$$\text{PDCAAS} = \text{EAAS} \times \text{\% IVPD}$$

Protein efficiency ratio. The protein efficiency ratio (PER) determines the effectiveness of a protein present in the sample. It was calculated based on Alsmeyer et al. (1974).

$$\text{PER}_1 = -0.684 + 0.456 \times \text{Leu} - 0.047 \times \text{Pro}$$
$$\text{PER}_2 = -0.468 + 0.454 \times \text{Leu} - 0.105 \times \text{Tyr}$$
$$\text{PER}_3 = -1.816 + 0.435 \times \text{Met} + 0.78 \times \text{Leu} + 0.211 \times \text{His} - 0.994 \times \text{Tyr}$$

Fatty acid analysis

The total lipids content obtained by hot extraction of uncooked and cooked mushroom flour was used to determine fatty acids methyl esters (FAMEs). The analysis was performed by the method outlined by Padua-Resurreccion & Benzon (1979).

Methylation. Methylation was performed by acid-catalysed method. The lipid sample in the screw cap glass tube (25×57 mm) was treated with acidified methanol (0.2 ml) (5% hydrochloric acid + 8.3 ml of acetyl chloride were added to 100 ml of absolute methanol in ice jacket). The

contents were vortexed and incubated (70°C, 10 hr). After cooling, distilled water (500 µl) and n-hexane (HPLC grade, 100 µl) were added, vortexed and allowed to separate. The n-hexane layer was aspirated into air-tight micro-centrifuge tubes and stored in refrigerator at 4°C for assay. The trans-esterified samples (100 µl) were made up to 1 ml by n-hexane and the fraction of samples (1 µl) was injected into the gas chromatograph.

Analytical parameters. The FAMEs were quantified by gas chromatography (GC-2010, Shimadzu, Japan) equipped with the fused silica column (BPX-70) and flame ionization detector (FID). The column was conditioned (10 hr) prior to use. The signal detected by FID was amplified and were processed in GC-solution software: http://www.shimadzu.eu/products/software//labsolutions/gcgcms/default.aspx. The analytical conditions were followed based on Nareshkumar (2007) (Table 2). The identification of peaks obtained from the lipid profiling was determined by comparing retention time, molecular weight and mass spectra with those available in NIST 11 (National Institute of Standards and Technology) library (NIST 11 mass spectrometry library; NIST/EPA/NIH; version # 2011).

Table 2 Analytical conditions for gas chromatography.

Auto-sampler settings
Injection sample volume 1 µl; terminal air gap, nil; number of rinses with solvent during per-run, 4; number of rinses with solvent during post-run, 6; number of rinses with sample, 5; washing volume, 8 µl; plunger suction and injection speed, high; syringe injection speed, low; injection port dwell time, 1 sec
Injection port settings
Injection mode, split; temperature, 225ºC; carrier gas, N2/air; pressure, 114.9 kPa; column flow, 1.29 ml/min; linear velocity, 34 cm/sec; purge flow, 3ml/min; split ratio, 50
Column oven settings
Initial temperature, 100ºC
Column oven temperature program
Equilibrium time, 3 min; total program time, 30 min
Temperatures hold time
100ºC, 1 min; 220ºC, 5 min; rate, 5ºC/min
Column information
Column, BPX-70; film thickness, 0.25 µm; inner diameter, 0.25 mm; column length, 30 m; column maximum temperature, 260ºC
Detector settings
Detector, FID, temperature, 280ºC; makeup gas, N2/air; makeup flow, 30 ml/min; H2 flow, 47 ml/min; airflow, 400 ml/min; sampling rate, 40 ms; stop time, 30 min; delay time, nil)

Data analysis

The *t*-test was followed to find out variation between uncooked cooked mushroom samples for different nutritional components based on Statistica Version # 8.0 (StatSoft 2008).

Results & Discussion

Analysis of proximal properties of food stuff is one of the basic steps which grossly reflect the nutritional value. The moisture content of *Amanita* powder was significantly higher in uncooked than cooked samples ($p<0.05$) (Table 3). Crude protein was significantly higher in cooked than uncooked samples ($p<0.05$). Total lipids content, ash content and calorific value were significantly higher in uncooked than cooked samples ($p<0.05$). There was no significant change in crude fibre and carbohydrate content between uncooked and cooked samples.

The increase of crude protein may be due to increased free amino acids owing to pressure cooking of *Amanita* sp., which has also reflected in increased amino acids. This view has been supported by Reid et al. (2016) based on studies carried out on *Amanita zambiana*, where crude protein significantly increased on microwave treatment and predicted that such change was due to increase in protein availability as a result of enzyme hydrolysis of insoluble protein. The crude protein content in *Amanita* sp. is higher than many edible *Amanita* spp. (*Amanita citrina, A, fluva,*

A. loosii and *A. rubescens*) (León-Guzmán et al. 1997, Tripathy et al. 2014, Sharma & Gautham 2015), while lower than *A. caesarea* and *A. zambiana* (Sharma & Gautham 2015, Reid et al. 2016).

Table 3 Proximal properties of uncooked and cooked *Amanita* sp. on dry weight basis (n=5; mean±SD; *t*-test: *p<0.05).

	Uncooked	**Cooked**
Moisture (%)	5.6±0.2*	4.8±0.4
Crude protein (%)	16.3±0.49	20±0.97*
Total lipids (%)	4.7±0.58*	1.9±0.65
Crude fibre (%)	7.4±0.37	6.7±0.43
Ash (%)	13.5±2.4*	8.2±2
Carbohydrates (%)	22.5±1.22	18.5±1.6
Calorific value (kJ/100 g)	827.4±25.8*	717.2±20.9

Usually the total lipid contents in edible mushrooms will be low and advantageous for human health. The total lipids in *Amanita* sp. is comparable to dried, but lower than fresh and frozen *A. zambiana* (Reid et al. 2016). The total lipids, crude fibre and ash contents of *Amanita* sp. is higher than *A. caesarea*, *A. citrina*, *A, fluva* and *A. rubescens* (León-Guzmán et al. 1997, Sharma & Gautham 2015). As seen in *Amanita* sp., the ash content decreases in mushrooms on conventional cooking as minerals drain away in water, thus alternative thermal processing helps retaining many essential minerals. The carbohydrates in uncooked *Amanita* sp. is higher than fresh and frozen *A. zambiana*, while lower than dried *A. zambiana* (Reid et al. 2016). The percentage of carbohydrate of *Amanita* sp. is lower than *A. caesarea*, *A. citrina* and *A. fluva* (Sharma & Gautham 2015). Although crude protein of *Amanita* sp. increased on cooking, total lipids as well as carbohydrates decreased, which has reflected in significantly low calorific value ($p<0.05$). The present study projects moderate quantities of protein as well as carbohydrates with low fat content in *Amanita* sp. thus this diet is helpful to maintain homeostasis in humans.

Table 4 Mineral composition of uncooked and cooked *Amanita* sp. (mg/100 g) (n=5; mean±SD; *t*-test: *p<0.01, **p<0.001; [a]NRC-NAS 1989 recommended pattern; BDL, below detectable level).

	Uncooked	**Cooked**	**Infants/ Children[a]**	**Adults[a]**
Sodium	855.3±10.1*	621.6±7.6	120-400	500
Potassium	4823±5.7**	3385±6.6	500-1600	1600-2000
Calcium	150±10*	94.3±4	600-800	800
Phosphorus	728±7.5*	618±15.7	500-800	800
Magnesium	127±2**	55.3±1.5	60-170	280-350
Manganese	134.3±4.5	BDL		
Iron	1306±5.56**	488.3±6.6	10	10-15
Sulphur	716.6±20.8**	248.3±4.7		
Copper	238.3±34	BDL	0.6-2	1.5-3
Aluminium	1231±10.2**	917.3±8.7		
Na-K ratio	0.17	0.20		
Ca-P ratio	0.18	0.15		

The uncooked and cooked samples of *Amanita* sp. were rich in potassium followed by iron and aluminium (Table 4). Sodium ($p<0.01$), potassium ($p<0.001$), calcium ($p<0.01$), magnesium ($p<0.001$), phosphorus ($p<0.01$), iron ($p<0.001$), sulphur ($p<0.001$) and aluminium ($p<0.001$) contents were significantly higher in uncooked than cooked samples. The rest of the minerals (copper and manganese) although detected in uncooked samples, they were below detectable limit

in cooked samples. Sodium, phosphorous and iron in uncooked *Amanita* sp. is higher, while calcium and magnesium contents are lower than *A. caesarea and A. loosii* (Tripathy et al. 2014).

The organic potassium salts in food have a broad range of health benefits for heart, kidney, bone and other tissues (Weaver 2013). Dietary potassium also lowers the risk of stroke by decreasing blood pressure (Weaver 2013). The food stuffs possessing Na-K ratio <1 are known to alleviate the high blood pressure (Yusuf et al. 2007). Sodium as well as potassium in *Amanita* sp. are higher than NRC-NAS (1989) stipulated standards for infants/children and adults. In addition, the Na-K ratio in uncooked as well as cooked samples is in favourable range (0.17-0.2). Calcium as the major component of bones confers hardness and rigidity. However, calcium content was higher in uncooked samples than cooked samples of *Amanita* sp., it was lower than NRC-NAS (1989) pattern. The phosphorus content was higher than NRC-NAS (1989) stipulated pattern, but the Ca-P ratio ranged between 0.18-0.15, which is not a favourable ratio (Shills & Young 1988). The magnesium content in uncooked *Amanita* sp. is comparable with NRC-NAS (1989) pattern for infants/children, but not for adults. Iron is an essential component for production of haemoglobin, which binds to oxygen. Iron content in uncooked and cooked *Amanita* sp. is higher than NRC-NAS (1989) pattern. Aluminium content was significantly lowered on cooking ($p<0.001$), which is an improvement sign to decrease its content by thermal treatments. According to Hill et al. (2000), lateritic rock/soil type is rich in iron as well as aluminium contents, thus *Amanita* sp. may also have these elements in high quantity.

Table 5 Amino acid composition of uncooked and cooked *Amanita* sp. in comparison with soybean, wheat and FAO-WHO pattern (g/100 g protein) (n=5, mean±SD; *t*-test: *p<0.05, ** p<0.01, ***p<0.001; [a]Bau et al. 1994, [b]USDA 1999, [c]FAO-WHO 1991 pattern, [d]Methionine + Cystine, [e]Phenylalanine + Tyrosine, [f]Ratio of total essential amino acids and total amino acids, BDL, below detectable level).

	Uncooked	**Cooked**	**Soybean[a]**	**Wheat[b]**	**FAO-WHO[c]**
Essential amino acid					
Histidine	1.76±0.11	2.5±0.03***	2.50	1.9-2.6	1.9
Isoleucine	3.73±0.28	4.73±0.03*	4.62	3.4-4.1	2.8
Leucine	7.17±0.06**	6.63±0.03	7.72	6.5-7.2	6.6
Lysine	9.38±0.03	9.04±0.03	6.08	1.8-2.4	5.8
Methionine	2.17±0.05	2.56±0.02**	1.22	0.9-1.5	2.5[d]
Cystine	0.08±0.01	0.19±0.02**	1.70	1.6-2.6	
Phenylalanine	3.32±0.04	4.74±0.05***	4.84	4.5-4.9	6.3[e]
Tyrosine	2.77±0.16	3.03±0.02**	1.24	1.8-3.2	
Threonine	4.32±0.16	4.75±0.04*	3.76	2.2-3.0	3.4
Tryptophan	BDL	BDL	3.39	0.7-1.0	1.1
Valine	5.05±0.04	5.92±0.06***		3.7-4.5	3.5
Non-essential amino acid					
Alanine	8.41±0.05	10.2±0.25**			
Arginine	7.11±0.03***	4.9±0.07			
Aspartic acid	7.21±0.05***	3.89±0.10			
Glutamic Acid	9.80±0.03	BDL			
Glycine	12.3±0.07 ***	9.83±0.05			
Proline	6.17±0.04	7.14±0.03***			
Serine	5.84±0.06	6.88±0.07***			
TEAA-TAA ratio[f]	0.41	0.50			

Among the EAA in *Amanita* sp., except for lysine ($p>0.05$) the rest were significantly changed on cooking (Table 5). In cooked *Amanita* sp., isoleucine, threonine ($p<0.05$), methionine, cystine, tyrosine ($p<0.01$), histidine, phenylalanine and valine ($p<0.001$) were significantly increased, while it was reverse for leucine ($p<0.01$). Except for cystine and tryptophan, rest of the

amino acids in uncooked/cooked *Amanita* sp. are comparable or surpassed the quantities present in soybean and wheat, so also the FAO-WHO (1991) stipulated pattern.

Among the non-essential amino acids, glycine was the highest followed by glutamic acid and alanine. Alanine ($p<0.01$), proline and serine ($p<0.001$) were significantly higher in cooked *Amanita* sp., while it was opposite for arginine, aspartic acid and glycine ($p<0.001$). The amino acid composition in *Amanita* sp. is higher than *A. caesarea*, *A. citrina*, *A. fluva* and *A. rubescens* (León-Guzmán et al. 1997, Sharma & Gautham 2015). Sudheep & Sridhar (2014) also reported significant increase in many amino acids on cooking the wild edible mushroom *Termitomyces globulus* of the Western Ghats. Lysine plays a key role in calcium absorption by reducing the amount of calcium excretion in urine. Its deficiency in chicks limits the synthesis of proteins (including cytokines) as well as proliferation of lymphocytes impairing immune responses leading to increased morbidity and mortality (Kidd et al. 1997, Konashi et al. 2000). Threonine is a major component of intestinal mucin and plasma γ-globulin in animals (Kim et al. 2007). Leucine has the capacity to dissolve visceral fat and helps in reduction of weight owing to activation of mTOR signalling pathway, which regulates protein synthesis and degradation in cells (Meijer & Dubbelhuis 2004). Increase in TEAA-TAA ratio on cooking *Amanita* sp. is favourable indication of the improved quality.

The IVPD was significantly higher in uncooked than cooked of *Amanita* sp. ($p<0.05$) indicates its nutritional value in uncooked stage (Table 6). According to the nutrition labelling regulations of the food and drug administration (FDA 1993), EAAS and PDCAAS determine the overall protein quality (Cuptapun et al. 2010). The EAAS of histidine, isoleucine, methionine + cystine, phenylalanine + tyrosine, threonine and valine were increased in cooked *Amanita* sp. The PDCAAS depicts protein quality of food stuffs, its range in *Amanita* sp. was from 57.9-103.6 and 53.7-90.7 in uncooked and cooked samples, respectively. The maximum PDCAAS value is

Table 6 *In vitro* protein digestibility (IVPD; *t*-test: *$p<0.05$), essential amino acid score (EAAS), protein digestibility corrected amino acid score (PDCAAS) and protein efficiency ratio (PER) of uncooked and cooked *Amanita* sp.

	Uncooked	**Cooked**
IVPD (%)	64.4±2.9*	53.7±3
EAAS		
Histidine	0.92	1.31
Isoleucine	1.33	1.68
Leucine	1.08	1.00
Lysine	1.61	1.55
Methionine + Cystine	0.90	1.10
Phenylalanine + Tyrosine	0.96	1.23
Threonine	1.27	1.39
Valine	1.44	1.69
PDCAAS		
Histidine	59.24	70.34
Isoleucine	85.65	90.21
Leucine	69.55	53.70
Lysine	103.68	83.23
Methionine + Cystine	57.96	59.07
Phenylalanine + Tyrosine	61.82	66.05
Threonine	81.78	74.64
Valine	92.73	90.75
PER		
PER_1	2.29	2.00
PER_2	2.49	2.22
PER_3	2.47	2.13

100% for milk, eggs, and soy protein, while those proteins devoid of EAA have a PDCAAS as 0. According to Friedman (1996), the PER of food stuffs greater than 2 are high quality, from 1.5-2 are moderate quality and less than 1.5 are poor quality. The PER of uncooked samples were greater than cooked *Amanita* sp. (2.29-2.49 vs. 2-2.22) indicates its high quality in uncooked as well as cooked stage.

Uncooked and cooked *Amanita* sp. showed highest quantity of palmitic acid, which significantly increased on cooking ($p<0.01$) (Table 7). Stearic acid was also significantly increased in cooked samples ($p<0.01$), while it was reverse for oleic acid ($p<0.001$). The TSFA were higher in cooked samples, while it was opposite for TUFA. Uncooked samples showed higher and favourable TUFA-TSFA ratio than cooked samples. In *Amanita* sp. total saturated fatty acids were higher but unsaturated fatty acids were lower than *A. rubescens* (León-Guzmán et al. 1997). Dietary stearic acid is well known for dramatic reduction of visceral adipose tissue (VAT) (Shen et al. 2014). Palmitic acid has many applications especially in cosmetics, detergents and emollient (Rabasco-Álvarez & González-Rodríguez 2000). Oleic acid regulates membrane lipid structure and in turn controls G protein-mediated signaling, which leads to reduction of blood pressure (Terés et al. 2008).

Table 7 Fatty acid methyl esters (FAMEs) of uncooked and cooked *Amanita* sp. (g/100 g lipid) (n=5, mean±SD; *t*-test: *p<0.01; **p<0.001).

	Uncooked	**Cooked**
Saturated fatty acid		
Palmitic Acid ($C_{16:0}$)	50.4±6.1	64±2.25*
Stearic Acid ($C_{18:0}$)	14.3±2	23.3±1.7*
Unsaturated fatty acid		
Oleic Acid ($C_{18:1}$)	23.3±1.5**	9.5±1.25
Total saturated fatty acids (TSFA)	64.7	87.3
Total unsaturated fatty acids (TUFA)	23.3	9.5
Ratio of TUFA-TSFA	0.36	0.10

Conclusions

This study addressed nutritional profile of traditionally consumed tender wild mushroom *Amanita* sp. occurring in the lateritic scrub jungles of southwestern India in uncooked and cooked stage. It is endowed with adequate protein, sufficient fibre, moderate quantity of carbohydrates and low total lipid content. Sodium, potassium and iron in uncooked as well as cooked *Amanita* sp. surpassed NRC-NAS (1989) recommended pattern with favourable Na-K ratio (<1). Except for cystine and tryptophan, the essential amino acids (EAA) are comparable or surpass the soybean and wheat, which fulfilled the FAO-WHO (1991) stipulated pattern. The total EAAS and total amino acid ratio has improved in cooked samples denotes its superiority. The *in vitro* protein digestibility was high in uncooked samples with good EAA score, protein digestibility corrected to amino acid score and favourable protein efficiency ratios (>2). Palmitic, stearic and oleic acids were the major fatty acid methyl esters in uncooked as well as cooked samples. The present study justified the value of traditional knowledge on nutritional advantages of tender sporocarps of *Amanita* sp. consumed by the tribals and local dwellers of southwestern India. Future studies on its bioactive potential and functional properties will open up possibilities of its utilization as food source or incorporation with other food stuffs to enhance the quality attributes.

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