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Amino acid profile of eighteen isolate of different edible macrofungal species

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ABSTRACT

Edible mushrooms from India (18 isolates belonging to 4 species) were profiled for protein, free and bound amino acids (AA). The protein content (range of 9.5-32.6%) was highest in *Pleurotus cintrinopileatus* and *P. sajor-caju*; free AA (range of 11.6-73.1 mg/g DW) was higher in *Hypsizygus tessulatus* and *Agrocybe aegerita*, bound AA (range of 57.4-171.9 mg/g DW) was also high in *H. ulmarius*, *P. djamor*, *P. florida*, *P. sajor-caju*. The essential free and bound AAs and chemical scores of isoleucine, tryptophan, phenylalanine was highest, higher in *Hericium erinaceus*, *P. cystidiosus*, *P. eryngi*, *P. sajor-caju*. The isoleucine (Ile) score in the free fraction of selected mushrooms were comparable or higher than the best five plant sources, while tryptophan (Trp) scores were almost double. Thus, these mushrooms are good sources of Ile, Trp and aromatic amino acids. The conditionally-essential and nonessential AAs were also quantified. This study reveals the diversity in protein and AA and nutritionally superior mushroom species.

Key words: Mushrooms, Nutrition, Free amino acids, Bound amino acids and Amino acid score

INTRODUCTION

Protein energy malnutrition (PEM) is an important public health concern among children in India which leads to a very high incidence of underweight, stunting and wasting. Verma and Prinja (2008) reported that intake of protein among 90% of the children was less than 80% of recommended dose in many regions of India. Edible mushrooms are increasingly being recognized for their high nutritional content and medicinal values. Mushrooms are protein dense food, also rich in minerals and fibre, and among the only vegetarian sources providing substantial vitamin D; in addition, they are low in carbohydrates and fat, and therefore low in calories. The therapeutic properties of mushrooms are attributed to its unique bioactive compounds such as heteropolysaccharides, statins *etc.* Worldwide production of cultivated mushrooms is estimated at 102.42 lakh tons and the leading countries are China, the US, the Netherlands and France (FAO, 2013). India currently produces around 0.982 lakh tons of mushrooms of which 85% are button mushroom. This scenario is however changing worldwide and in India as well due to the introduction of nutritionally and medicinally superior species which are geographically better suited like the Oyster, Shiitake, Milky and Paddy straw mushrooms. India produces about 98.4 million tons of surplus crop residues annually which are burnt causing environmental pollution (Jain *et al*., 2014). A mere 10% utilization of this surplus agro-waste to grow mushrooms can result in the production of 4.92 million tons of fresh mushrooms per annum, leading to the production of 123 thousand tons of protein per annum, which in turn can meet the protein requirement of 56 lakh people per annum, besides creating year-round employment for 2.02 million people and organic manure production to the tune of 5.9 million tonnes per annum (Pandey and Kumaran, 2018).

Amino acids are constituent building blocks of proteins, the latter having diverse structural and functional roles in the body. Deficiency of amino acids can therefore compromise normal health. The variety in the diet today ensures access to essential amino acids in most people, and deficiency symptoms are

rare. Of the 20 different amino acids required by the body to maintain good health, nine of these must be obtained from the diet as they cannot be synthesized by the human body, and therefore are essential amino acids. These are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. All these amino acids have specific roles and the deficiency symptoms also vary. Good dietary sources of essential amino acids are dairy, meat, eggs, nuts, seeds, whole grains *etc.,* thus plant- and animal-based foods contain essential amino acids. A healthy body can synthesize the other 11 amino acids, and therefore classified as the non-essential amino acids. Of these, arginine, cysteine, glycine, glutamine, proline serine, are conditionally essential in the human diet, where their synthesis can be limited under certain pathophysiological conditions. Amino acids occur in the free or the bound form in foods, where they form the building blocks of proteins.The objectives of this study were to profile and characterize the diversity in amino acid content in 18 indigenous isolates belonging to 14 species of edible oyster mushrooms collected across India, and identify superior species in terms of their protein and essential amino acids content which can help in the formulation of nutritionally superior and complete food combinations for vegetarians, and in addition help in reduction of consumption of meat

MATERIALS AND METHODS

Mushroom samples

The mushroom species/isolates used in this study are listed in Table 1. These mushrooms were collected, characterized, conserved and maintained at the germplasm repository of ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India. The mushrooms were cultivated on paddy straw or sawdust-basedspecies-specific substrates. The details of substrate requirement and environmental conditions maintained during growth are described in Table 2. Mushrooms after harvest were dehydrated at 48-50 °C in a commercial Tray dryer (5 kg capacity), for 14-16 h, to a moisture level of 10-11%, powdered in a laboratory blender and used for all nutrient assays.

Table 1. Mushroom species/ isolates studied for its protein and amino acid content

S. No.	ICAR-IIHR germplasm repository code	Scientific name
1	ICAR-IIHR-AA1	*Agrocybe aegerita*
2	ICAR-IIHR-BS1	*Hypsizygus tessulatus* (Brown coloured)
3	ICAR-IIHR-WS1	*H.tessulatus* (White coloured)
4	ICAR-IIHR-HU1	*H. ulmarius*
5	ICAR-IIHR-CA1	*Calocybe indica* - West Bengal isolate
6	ICAR-IIHR-CAJ	*Calocybe* spp. - Gujarat isolate
7	ICAR-IIHR-PCYST1	*Pleurotus cystidiosus*
8	ICAR-IIHR-DJ2	*P. djamor* - Karnataka, Western Ghats Shimoga pink coloured
9	ICAR-IIHR-DJ3	*P. djamor* - Madhya Pradesh (Pink coloured)
10	ICAR-IIHR-DJ4	*P. djamor* - Madhya Pradesh (White coloured)
11	ICAR-IIHR-PE1	*P. eryngi*
12	ICAR-IIHR-PE2	*P. eryngi*
13	ICAR-IIHR-PFL1	*P. florida*
14	ICAR-IIHR-PFL2	*P. floridanus* - Tamil Nadu isolate
15	ICAR-IIHR-YO1	*P. cintrinopileatus*
16	ICAR-IIHR-PSC1	*P. sajor-caju*
17	ICAR-IIHR-HE1	*Hericium erinaceus*
18	ICAR-IIHR-LE4	*Lentinula edodes*

Table 2. Cultivation conditions of different mushroom species/isolates

Cultivation conditions	All *Pleurotus* species, *Agrocybe aegerita*, *Hypsizygus ulmarius*	*Calocybe indica* (ICAR-IIHR-CA1) and *Calocybe* spp. (ICAR-IIHR-CAJ)	*Lentinula edodes*, *Pleurotus eryngi*, *Hypsizygus tessulatus*, *Hericium erinaceus*
Cultivation substrate	Shredded paddy straw having 65-67% moisture, filled @1 kg wet substrate per bag and sterilized at 121 °C, 18 lb pressure, 30 min		Hardwood sawdust supplemented with 25% rice bran, having 65-67% moisture, filled @1 kg wet substrate per bag and sterilized at 121 °C, 18 lb pressure, 120 min
Containers for growth	Polypropylene (PP) bags, (50 µm thick; 160 x 120 mm)		PP bags (50 µm thick; 120 x 100 mm)
Seed rate	5% of wet substrate		
Conditions for vegetative growth	Temperature 24-28 °C, Ambient humidity, No light	Temperature 30-38 °C, Ambient humidity, No light	Temperature 22-28°C, Ambient humidity, No light
Period of vegetative growth (days)	15-25 days (species dependent)	30-40 days	40-80 days (species dependent)
Conditions for mushroom formation	Slitting of bags, Temperature 24-28 °C (variety dependent), Humidity 80-85%, 12 h diffused natural or artificial light, Proper cross ventilation	Casing with pasteurized soil, Temperature 30-38 °C, Humidity 80-85%, 12 h diffused natural or artificial light, Proper cross ventilation	Cold water (10-12°C) shock treatment for *Lentinula edodes*, slitting of bags for other species, Temperature 18-20 °C, Humidity 80-85%, 12 h diffused natural or artificial light, Proper cross ventilation
Period of mushroom harvest (days)	6-20 days (species dependent) after bag slitting	20-25 days after casing	15-30 days after cold water shock treatment or slitting of bags
Total cropping period (seeding to last harvest) (days)	24-50 days (variety dependent)	65-70 days	60-110 days

Chemicals

The amino acid standards were purchased from Sigma Chemical Co. (USA), solvents used for liquid chromatography were of chromatographic/ MS grade, the reagents were of analytical grade, and Milli-Q (Millipore and system) water was used to prepare standards and mobile phases. Mobile phases were filtered through 0.45 µm pore size membranes before use.

Estimation of total free amino acids

The concentration of the total free and bound amino acids in the mushroomisolates was quantified by a modified method of Moore and Stein (1948). For the estimation of free amino acids, dried, homogenized mushroom sample (100 mg) was extracted in 5 ml of 80% ethanol. An aliquot of the extract was reacted with ninhydrin reagent, boiled for 20 min, cooled, and diluted with 1:1 v/v of n-propanol: water. After 15 min, the absorption was read at 570 nm in a spectrophotometer (PG Instruments T80+, UK). The amino acid concentration was calibrated against a phenylalanine standard (100 to 500 µg).

Estimation of total bound amino acids

Dried, homogenous mushroom sample (100 mg) was digested in 2 N KOH, followed by acid hydrolysis in 2 N HCl in boiling water bath; the excess acid was neutralized with KOH and an aliquot of this solution was subjected to amino acid estimation as described above (Moore and Stein, 1948).

Extraction of free amino acids for profiling

Dry samples of mushroom (0.5 g) were homogenized in 0.1% formic acid in 20% v/v methanol. The homogenate was diluted, filtered through 0.2 µm nylon filter membrane and 5 µl of the supernatant injected into UPLC-MS/MS (Nimbalkar *et al*., 2012).

Extraction of bound amino acids for profiling (Acid/Alkaline Hydrolysis):

Dry, homogenous samples of mushroom (0.5 g) were hydrolysed in 2 N KOH in a Thumburgs tube at 100 °C, for 6 h, followed by acid hydrolysis in 2 N HCl at 100 °C, for 6-8 h, under vacuum (McGrath, 1972). The mixture was cooled, diluted, an aliquot of the centrifuged supernatant was dried completely, dissolved in 5 ml of 0.1% formic acid in 20% methanol. This solution was filtered through 0.2 µm nylon filter membrane and 5 µl was injected into UPLC-MS/MS system.

UPLC-MS/MS conditions

An Acquity UPLC-H class coupled with TQD-MS/MS (Waters, USA) with ESI source and diode array detector was used for identification and quantification of amino acids. The methodology and for amino acid profiling was validated and from its linearity, detection and quantification limits and repeatability. The most sensitive detection mode, Multiple Reaction Monitoring (MRM), was employed (Fig.S1, Supplementary file). The operational parameters of the MS/MS system were optimized for each amino acid by direct sample infusion to select the most abundant mass-to-charge ratio (m/z); mass spectra were obtained at positive ionization mode (ES^+), where the full scan showed the most abundant forms of protonated $[M+H]^+$ amino acids molecules, and the same confirmed as precursor ions of the corresponding amino acids for the collision induced decomposition (CID) fragmentation. Based on the precursor ions and product ions, the MS-MS parameters - capillary voltage, extractor voltage and RF lens were set at 3.2 kV, 4 V and 0.1 V respectively; the gas flow for desolvation and cone was set at 650 and 50 L/h. The MRM variables used to calibrate the UPLC-MS/MS such as cone voltage and the collision energy for individual amino acid standards were optimized as described in Table 3.

Table 3. MRM of amino acids standards

Sl. No.	Compounds	Formula/ Mass	Parent ion (m/z) $[M+H]^+$	Daughters	Cone voltage (V)	Collision energy (eV)	Ion mode
1	Asparagine	132.00	133.03	74.02	8	16	ES+
2	Aspartic Acid	133.00	134.03	74.02	8	14	ES+
3	β-3,4-Dihydroxy phenylalanine	197.20	198.10	152.06	10	15	ES+
4	Citrulline	175.01	176.04	70.04	8	22	ES+
5	Cysteine	121.03	122.00	75.99	26	15	ES+
6	Alanine	89.10	90.13	44.09	10	8	ES+
7	Arginine	174.00	175.03	70.04	14	20	ES+
8	Ethionine	163.02	164.12	56.06	10	15	ES+
9	Methionine	148.98	150.01	104.02	8	10	ES+
10	Proline	115.05	116.02	70.03	12	12	ES+
11	Threonine	119.11	120.08	74.09	8	10	ES+
12	Glutamic Acid	147.03	148.06	84.04	12	12	ES+
13	Glycine	75.01	76.17	30.08	10	12	ES+
14	Histidine	155.06	156.22	110.14	12	12	ES+
15	Isoleucine	131.18	132.21	86.15	10	10	ES+
16	Leucine	131.18	132.15	86.08	10	10	ES+
17	Lysine	146.13	147.16	84.13	8	14	ES+
18	Norleucine	131.11	132.14	86.14	8	8	ES+

19	Norvaline	117.11	118.08	72.09	8	8	ES+
20	Phenylalanine	165.00	166.10	120.13	10	12	ES+
21	Serine	105.04	106.07	60.10	8	12	ES+
22	Tryptophan	204.11	205.14	188.09	10	12	ES+
23	Tyrosine	181.11	182.14	136.19	8	14	ES+
24	Valine	117.22	118.06	72.15	8	8	ES+

The mobile phase consisted of the aqueous Solvent A, 0.1% formic acid in water, and the organic Solvent B, methanol: water (50:50) with 0.1% formic acid, with a gradient elution program as detailed in Table 4, at a flow rate of 0.1 mL/min. The analytical column was 2.1x50 mm UPLC BEH C18 column (Waters) of 1.7 µm particle size, protected by a vanguard BEH C18 with 1.7 µm. The column temperature was maintained at 25 °C. The eluted amino acid was monitored directly without any split in the TQD-MS/MS (Waters, USA) system, optimized for the amino acids analysis.

Table 4. LC-MS gradient elution program for amino acid profiling

Time (min)	Aqueous mobile phase A (%)	Organic mobile phase B (%)
0	98	5
1	95	5
10	80	20
11	80	20
15	60	40
15.5	60	40
19	95	5
20	95	5

Statistical analysis

Quantification of total free and bound amino acids and their component amino acids in 18 oyster mushroom isolates were carried out in triplicates. The data were analysed by one-way analysis of variance (ANOVA). Tests of significant differences were determined by Duncan's multiple range tests at $p<0.05$. Multivariate analysis was carried out by applying principal component analysis.

RESULTS AND DISCUSSION

In this study, it was possible to establish the significant variability among the 18 mushroom isolates in their content of total protein, free and bound amino acids (Table 5), as also in the amino acid profile of the free and bound amino acids.

Protein content:

The protein content was obtained from nitrogen using 6.25 as the conversion factor, on dry weight basis; it ranged between 9.51 to 32.61%. The genus *Calocybe* showed lowest protein values of 9.51 and 14.5% in *Calocybe indica* (IIHR-CA1) and *Calocybe* spp. (IIHR-CAJ) respectively. The protein values of this genus also showed variability with the isolate IIHR-CAJ from Gujarat showing higher protein content as compared to IIHR-CA1 from West Bengal. Among the isolates of the genus *Pleurotus, P. cintrinopileatus* (IIHR-YO1) and *P. sajor-caju* (IIHR-PSC1) contained the highest protein content of 32.61 and 31.78% respectively. Among *P. djamor* isolates, the pink isolate from Madhya Pradesh (IIHR-DJ3) showed significantly higher protein content (28.7%) compared to the pink isolate from Western Ghats - IIHR-DJ2 – (20.62%). The white isolate from Madhya Pradesh (IIHR-DJ4) had intermediate protein content (24.16%). There was significant difference in the protein content of the two isolates of *P. eryngi,* IIHR-PE1 (20.51%) and IIHR-PE2 (16.55%). The variation in protein content among

Hypsizygus species was statistically significant with 25.63% in *H.ulmarius* (IIHR-HU1) compared to 24.55% in brown isolate of *H.tessulatus* (IIHR-BS1) and 20.47% in white isolate (IIHR-WS1). Among the six genera studied, the genus *Pleurotus* had the highest protein content followed by the genus *Agrocybe* and *Lentinula*. All the mushrooms used in the present study were grown under similar lab conditions. Hence the variations occurring in the protein content across the genera, species and isolates of the same species can be attributed to both the genetic makeup of the isolate and the external growing substrates.

Free and bound amino acids:

In foods, amino acids are present in both the free and the bound forms (where it is present as building blocks of proteins). The free and bound amino acid content varied significantly among the 18 isolatesof mushroom studied, and the total bound amino acids content was almost 3.6 times higher than that of free amino acids content (Table 5). Content of total free amino acids was highest in *H. tessulatus*, (IIHR-BS1 and IIHR-WS1), these isolates were different only in their colour (i.e.) brown and white respectively,and *A.aegerita* (IIHR-AA1). It was least in *P.djamor* (IIHR-DJ2 and IIHR-DJ3), both pink coloured isolates, collected from different states of India – Western Ghats of Karnataka and Madhya Pradesh respectively. As observed in the content of free amino acids, the total bound amino acids content was also higher in *H. tessulatus* isolates IIHR-BS1 and IIHR-WS1, and *A. Aegerita* (IIHR-AA1). It was high in *H. ulmarius* (IIHR-HU1) also. Both free and bound amino acids were high also in *P. florida* (IIHR-PFL1)and *P. sajor-caju* (IIHR-PSC1). The *Calocybe* spp (IIHR-CAJ) recorded the least free and bound amino acid content. But in contrast to the content of free amino acid, *P. djamor* from Western Ghats of Karnataka IIHR-DJ2 (151.32 mg Phe eq./ g DW) and Madhya Pradesh IIHR-DJ3 (168.42 mg Phe eq./ g DW) (both pink coloured isolates)had significantly higher bound amino acids. *L.edodes* (IIHR-LE4), had low contents of free and bound amino acids.

In a study in developing mutant rice seed with enhanced protein and grain Lys, Schaeffer and Sharpe (1997) found some free amino acids of developing seed to be inversely correlated to total amino acids in proteins of the mature grain. Asp, Thr, Met and Lys were enhanced in protein, but were conspicuously lower in the free amino-acid pool, probably due to the mutant-developing grains processing Asp more rapidly than control. Conversely, Arg, Val and Glu/ Gln accumulated as free amino acids more in mutants than in control. In this study, we were unable to draw such correlations.

Table 5. Total protein, free and bound amino acid contents in 18 species/ isolates of mushrooms

Sl. No.	ICAR-IIHR germplasm repository code	Scientific name	N (%)	Protein (%)	Free amino acids$	Bound amino acids$
1	ICAR-IIHR-AA1	*Agrocybe aegerita*	4.18	26.13	67.95^{ab}	168.44^{a}
2	ICAR-IIHR-BS1	*Hypsizygus tessulatus* - Brown isolate	3.93	24.55	73.11^{a}	169.50^{a}
3	ICAR-IIHR-WS1	*H. tessulatus* - White isolate	3.28	20.48	65.91^{b}	165.19^{ab}
4	ICAR-IIHR-HU1	*H. ulmarius*	4.06	25.36	55.49^{c}	171.85^{a}
5	ICAR-IIHR-CA1	*Calocybe indica* - West Bengal isolate	1.52	9.51	19.75^{fg}	63.26^{h}
6	ICAR-IIHR-CAJ	*Calocybe* spp. - Gujarat isolate	2.32	14.50	22.11^{fg}	57.41^{h}
7	ICAR-IIHR-PCYST1	*Pleurotus cystidiosus*	2.82	17.64	23.31^{f}	96.91^{e}
8	ICAR-IIHR-DJ2	*P. djamor* - Karnataka, Western Ghats Shimoga pink coloured	3.30	20.63	11.56^{h}	151.32^{c}

9	ICAR-IIHR-DJ3	*P. djamor* - Pink isolate Madhya Pradesh	4.59	28.70	12.21^{h}	168.42^{a}
10	ICAR-IIHR-DJ4	*P. djamor* - White isolate Madhya Pradesh	3.87	24.16	37.97^{e}	87.74^{gf}
11	ICAR-IIHR-PE1	*P. eryngi*	3.28	20.51	24.13^{f}	132.48^{d}
12	ICAR-IIHR-PE2	*P. eryngi*	2.76	17.24	39.17^{e}	96.94^{e}
13	ICAR-IIHR-PFL1	*P. florida*	4.04	25.24	35.88^{e}	164.08^{ab}
14	ICAR-IIHR-PFL2	*P. floridanus* - Tamil Nadu isolate	4.77	29.80	16.70^{hg}	146.19^{c}
15	ICAR-IIHR-YO1	*P. cintrinopileatus*	5.22	32.61	22.58^{fg}	128.83^{d}
16	ICAR-IIHR-PSC1	*P. sajor-caju*	5.09	31.79	45.86^{d}	159.22^{b}
17	ICAR-IIHR-HE1	*Hericium erinaceus*	3.89	24.33	47.30^{d}	91.98^{ef}
18	ICAR-IIHR-LE4	*Lentinula edodes*	4.26	26.65	18.58^{fg}	82.38^{g}
Mean			3.73	23.32	35.53	127.90
Range			1.52-5.22	9.51-32.61	11.56-73.11	57.41-171.85
SEd			0.468	5.62	7.16	
CD (0.05)				0.934		
CV%				3.18		

$ in (mg Phe eq./ g DW)

Amino acid profile

The profile of the free and bound amino acid in the 18 mushroom isolates was analysed. The essential amino acids, histidine (His), isoleucine (Ile), Leucine (Leu), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), valine (Val) and lysine (Lys), available in the free amino acid fraction one given in supplementary data. It was higher in *A. aegerita* (IIHR-AA1) and *P. sajor-caju* (IIHR-PSC1), followed by *H. ulmarius* (IIHR-HU1 and *H. erinaceus* (IIHR-HE1); while it was least in *L. edodes* (IIHR-LE4) and *H. tessulatus* (IIHR-WS1, a white coloured isolate). From the mean values it can be seen that Ile was present in the highest amount (1194.29 mg/100 g DW), followed by Trp (267.48 mg/ 100 g DW), Phe (91.5 mg/100 g DW); Leu and Tyr were at par (24.11, 21.24 mg/100 g DW respectively). The remaining essential amino acids were present in low quantities and at par supplementary data available online.

A. aegerita (IIHR-AA1) had the highest Ile content (2429.11 mg/100 g DW) among the 18 isolates of mushroom studied, followed by *H. ulmarius* (IIHR-HU1) (1950.2 mg/100 g DW), and least in *L. edodes* (IIHR-LE4) (535.24 mg/100 g DW). Trp was more in IIHR-PSC1 (852.8 mg/100 g DW) and IIHR-HE1 (827.1 mg/100 g DW), followed by IIHR-PCYST1 (580.8 mg/100 g DW) and IIHR-PE2 (529.6 mg/100 g DW); and least in IIHR-DJ2, IIHR-DJ3, IIHR-DJ4, IIHR-PFL1, IIHR-HU1. Phe was highest in IIHR-AA1 (161.87 mg/100 g DW), followed by IIHR-HU1 (139.1 mg/100 g DW) and IIHR-PSC1 (133.87 mg/ 100 g DW), and least in IIHR-LE4. Leu was high in IIHR-BS1 (82 mg/100 g DW), and least in IIHR-CA1, (2.4 mg/100 g DW). Tyr was highest in IIHR-PE2 (72.47 mg/100g DW), and least in IIHR-LE4. The contents of His, Thr, Lys and Val were negligible (mean values of 2.31, 6.19, 6.61 and 7.75 mg/ 100 g DW respectively).

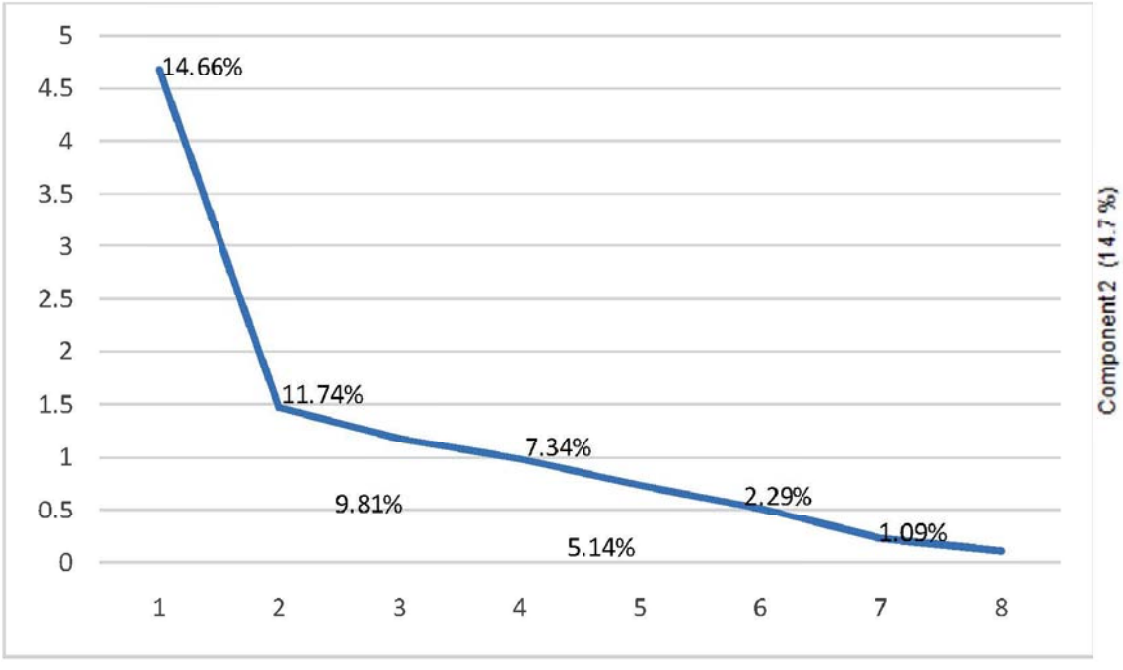
The principal component analysis (PCA) revealed that four principal components (PCs) accounted for 82.98% of the total variability in free, essential amino acid content in the 18 mushroom species/ isolates (Fig. S2, Supplementary file). The first PC had the

highest Eigen value (4.68), and accounted for 46.77% of the total variability in the whole data set, while the second PC had Eigen value of 1.47 and accounted for 14.66% of the total variability, and the third PC contributed 11.74% to the total variability. The next four PCs contributed 9.81 to 2.29% to the total variability and the remaining three PCs had <1 Eigen values, and accounted for <2.26% variability and are therefore insignificant. The components PC1 and PC2, together accounting for 61.4% of variability, were plotted to reveal eight distinct groups (Fig 1). *A.aegerita*, AA (IIHR-AA1) and *P. sajor-caju*, Psc (IIHR-PSC1) with the highest free essential amino acid contents, followed by *Hysizygus ulmarius*, Hu (IIHR-HU1) occupied distinct groups in I quadrant. The mushroom isolates with moderately high free amino acid content, *H. erinaceus*(IIHR-HE1),*H. tessulatus* (IIHR-BS1,brown coloured) and*H. tessulatus* (IIHR-WS1,white coloured) formed a distinct group, as also *P. djamor* (IIHR-DJ2 and IIHR-DJ3, bothpink coloured) and *P. djamor* (IIHR-DJ4,white coloured), *P. florida* (IIHR-PFL1) and*P. citrinopileatus* (IIHR-YO1). Mushrooms with high contents of specific amino acids, such as *P. floridanus* (IIHR-PFL2) (high Thr, Phe, Trp and Tyr contents) and *P. eryngi* (IIHR-PE2) (high Trp, Tyr and Leu contents) formed independent groups. The mushrooms with low contents of most free essential amino acids *viz*. *L. edodes* (IIHR-LE4), *P. eryngi* (IIHR-PE1) and *P. cystidiosus* (IIHR-PCYST1) (though high in Trp), and *Calocybe* spp. (IIHR-CAJ) and *Calocybe indica* (IIHR-CA1) grouped separately.

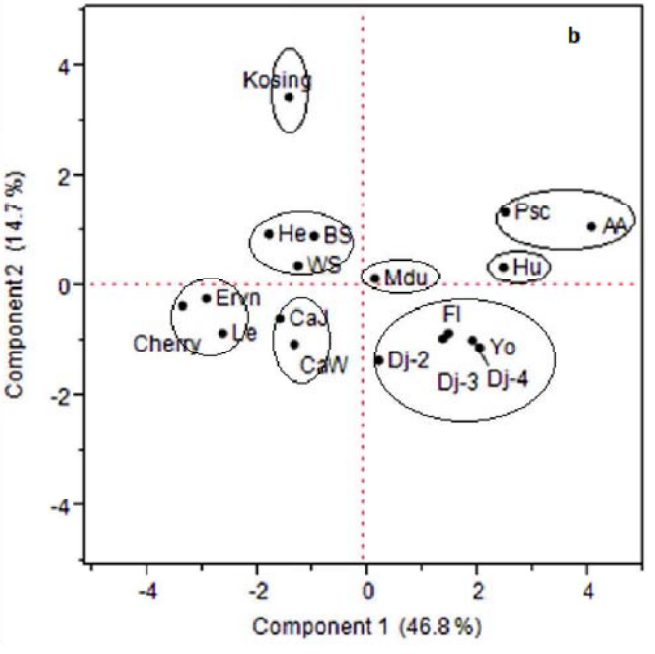
These values were computed against the essential amino acid score recommended by FAO (2013) for children, adolescents and adults, to obtain the amino acid scores of individual mushroom isolates, for each essential amino acid The free amino acid scores were higher in IIHR-HE1, IIHR-PCYST1 and IIHR-PE2, followed by IIHR-PSC1; it was least in IIHR-YO1 and IIHR-PFL1. These mushroom isolates had higher scores of Trp and Ile, with mean values of 182.7 and 174.4% respectively; followed by the aromatic amino acids (Phe+Tyr) 12.6%. Trp was highest in IIHR-HE1 (515%), IIHR-PCYST1 (498.9%), IIHR-PE2 (465.4%) and IIHR-PSC2 (404.5%), but not detectable in IIHR-DJ2, IIHR-DJ3, IIHR-DJ4 and IIHR-PFL1. Ile was high in IIHR-CA1 (312%), IIHR-AA1 (309.9%), IIHR-HU1 (256.3%) and IIHR-DJ4 (211.1%); the aromatic amino acids were also high in IIHR-CA1 (27.5%) and IIHR-AA1 (17.7%), and in IIHR-PE2 (21.7%) additionally. Both Ile and the aromatic amino acids were least in IIHR-LE4. When compared to the FAO amino acid scores, the mean values of the free essential amino acids of the mushrooms could provide over 27 times the recommended scores of Trp and almost 6 times that of Ile, 30% of aromatic amino acids, and 1-8% of the remaining free essential amino acids. Thus, the mushrooms studied are promising vegan sources of essential amino acids.

Of the conditionally essential free amino acids, Asn content was higher, and among the isolates IIHR-PSC1 (286.5 mg/100 g DW), followed by IIHR-PFL2 and IIHR-AA1 had higher contents of Asn. Of the non-essential amino acids, Ala (18.58 mg/100 g DW), followed by Glu were in higher content (7.19 mg/100 g DW); more in IIHR-AA1 and IIHR-BS1 respectively.

As in the free amino acids, Ile content (mean of 341.92 mg/100 g DW) was higher among the essential bound amino acids also, though the former was 3.5 times more than the latter (Table 9). The other amino acids in high content included Phe (129.8 mg/100 g DW), Trp (63.9 mg/100 g DW) and Leu (59 mg/100 g DW). These amino acids were also high in the free fraction. Ile was more in IIHR-BS1, IIHR-AA1, IIHR-WS1 (703.6, 556.2, 539.8 mg/100 g DW respectively) and less in IIHR-PSC1 and IIHR-CAJ (121.7, 169.69 mg/100 g DW respectively); Phe was high in IIHR-HU1, IIHR-AA1 and IIHR-LE4 (170 to 176 mg/100 g DW), and again low in IIHR-PSC1 and IIHR-CAJ (78.9, 84.98 mg/100 g DW respectively). Trp content was high only in IIHR-BS1 (1123 mg/100 g DW), and negligible in the other species. Bound Leu was high in IIHR-AA1 and IIHR-LE4 (211, 154.9 mg/100 g DW respectively), but low in IIHR-PSC1, IIHR-YO1 and IIHR-CAJ (17.52, 16.26 and 19.34 mg/100 g DW respectively). IIHR-BS1 and IIHR-AA1 had higher bound amino acid content; while IIHR-CAJ and IIHR-PSC1 had the least among the 18 mushroom isolates studied.

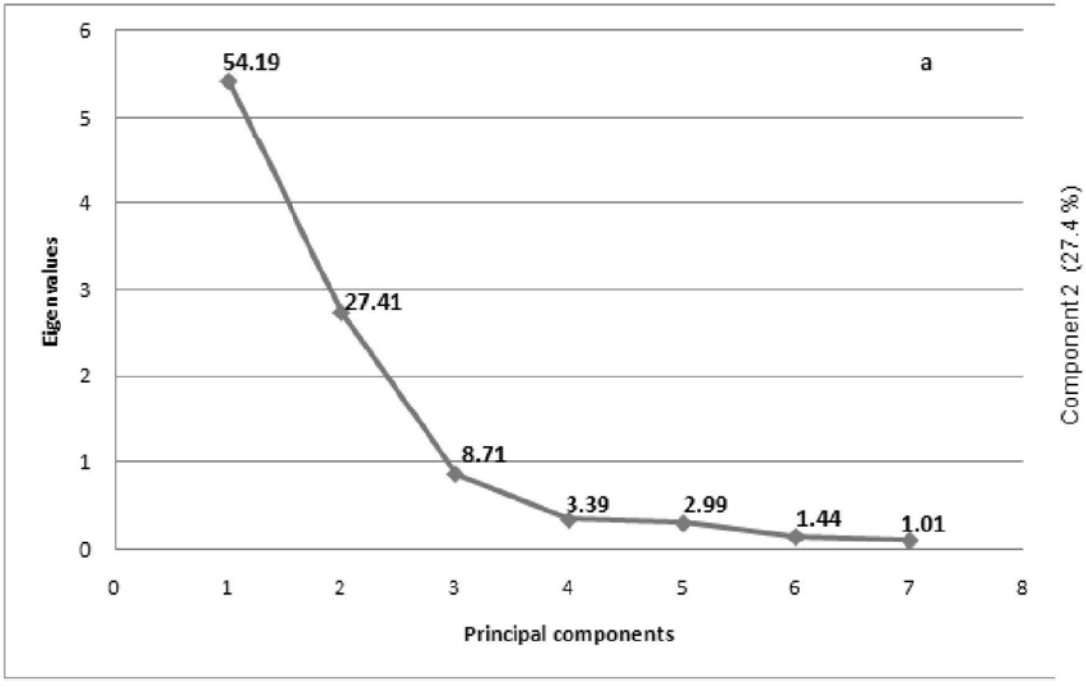


2a. Given value of major components for different oyster mushroom isolates

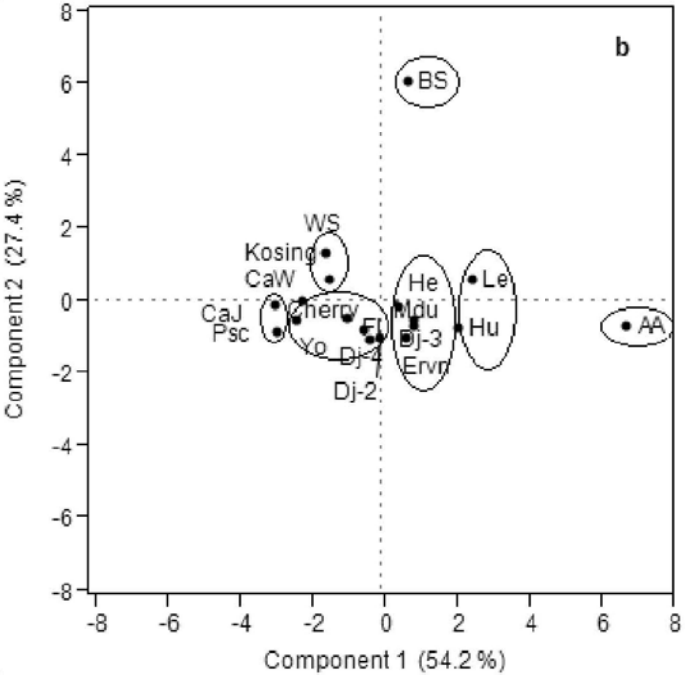


2b. Components score plot for PC1 and PC2 for free, essential amino acids in 18 Oyster mushroom / isolates

Code	Species/ isolates	Code	Species/ isolates	Code	Insert species isolates
He	*Hericium erinaceus* (IIHR-HE1)	CaJ	*Calocybe* spp. Gujarat isolate (IIHR-CAJ)	Hu	*Hypsizygus ulmarius* (IIHR-HU)
BS	Brown *Hypsizygus tessulatus* (IIHR-BS1)	CaW	*Calocybe indica* West Bengal isolate (IIHR-CA1)	Fl	*Pleurotus florida* (IIHR-PFL1)
WS	White *Hypsizygus tessulatus* (IIHR-WS1)	Kosing	*Pleurotus eryngi* (IIHR-PE2)	Dj2	*Pleurotus djamor*- Pink-Western ghats isolate (IIHR-DJ2)
Ervn	*Pleurotus eryngi* (IIHR-PE1)	Mdu	*Pleurotus floridanus* (IIHR-PFL2)	Dj3	*Pleurotus djamor*- Pink-Madhya Pradesh isolate (IIHR-DJ3)
Cherry	*Pleurotus cystidiosus* (IIHR-PCYST-1)	Psc	*Pleurotus sajor-caju* (IIHR-PSC-1)	Dj4	*Pleurotus djamor*- White-Madhya Pradesh isolate (IIHR-DJ4)
Le	*Lentinula edodes* (IIHR-LE4)	AA	*Agrocybe aegerita* (IIHR-AA1)	Yo	*Pleurotus citrinopileatus* (IIHR-YO1)



3a. Eigenvalue of major components for different mushroom / isolates



3b. Components score plot for PC1 and PC2 for bound, essential amino acids in 18 Oyster mushroom / isolates

Code	Species/ isolates	Code	Species/ isolates	Code	Species/ isolates
He	*Hericium erinaceus* (IIHR-HE1)	CaJ	*Calocybe* spp. Gujarat isolate (IIHR-CAJ)	Hu	*Hypsizygus ulmarius* (IIHR-HU)
BS	Brown *Hypsizygus tessulatus* (IIHR-BS1)	CaW	*Calocybe indica* West Bengal isolate (IIHR-CA1)	Fl	*Pleurotus florida* (IIHR-PFL1)
WS	White *Hypsizygus tessulatus* (IIHR-WS1)	Kosing	*Pleurotus eryngi* (IIHR-PE2)	Dj2	*Pleurotus djamor*- Pink-Western ghats isolate (IIHR-DJ2)
Ervn	*Pleurotus eryngi* (IIHR-PE1)	Mdu	*Pleurotus floridanus* (IIHR-PFL2)	Dj3	*Pleurotus djamor*- Pink-Madhya Pradesh isolate (IIHR-DJ3)
Cherry	*Pleurotus cystidiosus* (IIHR-PCYST-1)	Psc	*Pleurotus sajor-caju* (IIHR-PSC-1)	Dj4	*Pleurotus djamor*- White-Madhya Pradesh isolate (IIHR-DJ4)
Le	*Lentinula edodes* (IIHR-LE4)	AA	*Agrocybe aegerita* (IIHR-AA1)	Yo	*Pleurotus citrinopileatus* (IIHR-YO1)

PCA revealed three PCs accounting for 90.31% of the total variability in bound, essential amino acid content in the 18 mushroom isolates (Fig. S3, Supplementary file). The first PC had the highest Eigen value (5.42), resulting in 54.19% of the total variability, the second PC with 2.74 Eigen value accounting for 27.41% to the total variability, and the third PC contributed 8.71% to the total variability. The next two PCs contributed 3.39 and 2.99% to the total variability and the remaining five PCs had <2 Eigen values, and accounted for <3.31% variability and are therefore insignificant. The components PC1 and PC2, together accounting for 81.6% of variability, were plotted to reveal eight distinct groups (Fig.2). BS (IIHR-BS1) with the highest bound essential amino acid and the only mushroom of the 18 isolates with considerable Trp contents formed a separate group in I quadrant; *A. aegerita* (IIHR-AA1) with the next highest content also formed a separate group. *L. edodes* (IIHR-LE4) and *H. ulmarius* (IIHR-HU1) formed a distinct group, *L.edodes* (IIHR-LE4) was high in Phe and Leu, and *H. ulmarius* (IIHR-HU1) was high in Phe and Lys. The mushrooms with the next highest contents, were *H. tessulatus* (IIHR-WS1) with high Ile and *P. eryngi* (IIHR-PE2) with high Ile and Trp formed a separate group. The mushroom isolates with moderately high bound amino acid content, *H. erinaceus* (IIHR-HE1), *P. floridanus* (IIHR-PFL2), *P. djamor* (IIHR-DJ3) and *P. eryngi* (IIHR-PE1) formed a distinct group. The isolates with lesser contents *P. florida* (IIHR-PFL1), *P. djamor* (IIHR-DJ2), *P. djamor* (IIHR-DJ4), *P. cystidiosus* (IIHR-PCYST1), *C. indica* (IIHR-CA1) and *P. citrinopileatus* (IIHR-YO1) grouped together, though *P. citrinopileatus* was high in Trp. *Calocybe* spp. (IIHR-CAJ) and *P. sajor-caju* (IIHR-PSC1) with lowest contents of these amino acids grouped together.

IIHR-AA1 and IIHR-HU1 were high in both free and bound essential amino acids, while IIHR-CAJ and IIHR-CA1 were both low. IIHR-PE2 had high Trp contents in both free and bound amino acids. IIHR-LE4 which was low in free essential amino acids was high in bound essential amino acids, and the reverse was true of IIHR-PSC1 mushroom. IIHR-PFL1, IIHR-DJ-2 and IIHR-DJ4 grouped together in both free and bound amino acids; but unlike in free amino acids, IIHR-HE1, IIHR-BS1 and IIHR-WS1 were in distinct groups in bound amino acids.

Score of bound amino acids of Ile was higher, followed by the aromatic amino acids Phe and Tyr, and marginal scores of Leu and Met (Table 10). Ile was high in IIHR-BS1, IIHR-WS1, IIHR-CA1 and IIHR-PE2 (82 to 96%); Met score was high in IIHR-DJ-2, IIHR-AA1, IIHR-HU1, IIHR-PE1 (1.6 to 1.9%); while *H. tessulatus* (IIHR-BS1 and IIHR-WS1), IIHR-YO1 and IIHR-PSC1 had low scores of Met. Leu score was highest in IIHR-AA1 (13.2%), followed by IIHR-LE4 (9.5%); IIHR-YO1 (0.8%) and IIHR-PSC1 (0.9%) had low scores. When compared to the FAO amino acid scores, the mean values of the bound essential amino acids of the mushrooms could provide over 1.8 times the recommended scores of Ile and 37% of aromatic amino acids, 12% of Trp, and 5-7% of Leu and Met; the scores of the remaining amino acids was insignificant.

Kayoden *et al*. (2015) have reported that the chemical score of essential amino acids of the commercially cultivated oyster mushroom (*P. sajor-caju*), ranged from 55.9% Met to 150.3% Ile, and this was comparable to standard dietary reference intake requirement. In our *P. sajor-caju* isolate (IIHR-PSC1), the Ile score was comparable at 186.5%, but the Met score was very low (3.7%). Oyetayo *et al.* (2007) analysed the cap and stalk of two cultivated *P. sajor-caju* varieties obtained from the wild, where they observed that the cultivated mushroom cap accumulated more crude protein than the wild. The mushroom was a rich source of the essential amino acid Leu, while Met and Cys chemical scores were low, probably the reason for the low Met content in IIHR-PSC1.

Manzi *et al.* (1999) have compared the nutritional content in several isolates of edible mushrooms, *P.ostreatus, P. eryngii, P. pulmunarius* and *L. edodes.* They have reported total nitrogen ranging from 3.47 to 7.93% (dry basis), (values comparable to this study), with *P. ostreatus* isolates exhibiting the largest variability. They reported that in *P. pulmunarius* and *L. edodes*, Glu (12.8%), Asp (9.1%) and Arg (3.7%) were the most abundant amino acids, but with low Arg content. The chemical score ranged between 96 to 110%, the limiting amino acid being Leu and/ or Lys. In this study also Leu and Lys scores were low, as also His, Met and Thr. Thus, the amino acid composition varies significantly among mushroom isolates.

Musieba *et al*. (2013) reported the proximate composition and amino acids contents of indigenous *P. citrinopileatus* collected from Kakamega forest in Western Kenya, which is an integral part of their traditional food system; eight essential amino acids have been reported in decreasing order of abundance: Leu > Val > Thr > Lys > Phe > Ile > Met > Trp. The non-essential Glu was also present in high proportion. The results presented here vary much from their report. The variability of amino acids in white button mushrooms *(Agaricus bisporus)* and oyster mushrooms (*Pleurotus pulmonarius*) was reported by Rana *et al*. (2015). Soluble protein content of *A. bisporus* and *P. pulmonarius* was 3.3% and 1.8%, respectively, the *Pleurotus* isolates studied here had much higher protein content. *A. bisporus* contained nine essential amino acids, while *P. pulmonarius* contained only five, among the 17 amino acids identified by thin layer chromatography. In an early study by Zakia *et al*. (1963) *Pleurotus* spp. had 2.8% protein and 0.1% non-protein nitrogen on freshweight basis. Seventeen amino acids were identified, including all the essential amino acids, with all except Met and Phe being present in high concentration.

Sharma *et al*. (2012) studied five wild edible species of *Lentinus*, *L. sajor-caju*, *L. connatus*, *L. torulosus*, *L. cladopus* and *L. squarrosulus* from Northern India. Asp, the predominant amino acid and Pro which were more in *L. squarrosulus*, Arg and Ala were maximum in *L. torulosus*, Tyr was maximum in *L. cladopus*.

A study by Jaworska and Bernas (2013) demonstrates how processing treatments affect chemical composition of mushrooms. Compared to fresh mushrooms, most amino acids except Gln and Ile were lesser in frozen mushrooms. The decrease was more in *A. bisporus* soaked and blanched in citric and L-ascorbic acid solutions, and in *Boletus edulis*, soaked and blanched in pectin solution. Blanching treatment alone resulted in higher Asn, Gln, Ile and Lys than those soaked and blanched.

Of the conditionally essential bound amino acids, Pro and Ser were present marginally and of the non-essential amino acids Glu and Ala, as reported above for free amino acids. Pro was high in IIHR-AA1, IIHR-PCYST1, IIHR-CA1, IIHR-PFL2 (10 to 16mg/ 100 g DW); Ser was more in IIHR-AA1, IIHR-HU1 (>6mg/100 g DW). The non-essential amino acids were high in IIHR-BS1, IIHR-AA1and IIHR-LE4. These amino acids were less in IIHR-WS1and IIHR-CAJ (Table 11). The non-proteinogenic amino acids like ethionine, citrulline, β-3,4-dihydroxy phenylalanine were negligible in all the mushrooms studied.

High protein foods, such as tofu and quinoa, contain significant amounts of essential amino acids. A comparison was made of the amino acid scores reported from the five best plant sources (from FAO data compiled from among 50 plant sources), to the free and bound amino acid scores in selected mushroom isolates used in this study, rich in essential amino acids (Table 12). The best plant sources were soybean, cowpea, quinoa, turnip seed and sword bean, with protein content ranging between 6.6-38.0%, while in the mushrooms the protein content ranged between 9.51-31.79%. These mushrooms had significantly higher Ile and Trp contents. The Ile score, especially in the free fraction of selected mushrooms (66-312%) were comparable or higher than the best five plant sources (152-186%); though Ile was lower in the bound fraction (12-96%). Similarly, Trp scores in the plant sources ranged between 104-264%, while in the free fraction of a few mushrooms it was almost twice as much, in IIHR-HE1 it was as high as 515%; the content in the bound fraction was much lower. The total aromatic amino acids score was ~1/10th of the plant sources listed. However, the scores of the remaining amino acids were not appreciably high. Thus, the mushrooms listed in table 12 are an excellent source of Ile in the free fraction (IIHR-CA1, IIHR-AA1, ICAR-IIHR-HU1), good source of Trp (IIHR-HE1, IIHR-PCYST1, IIHR-PE2, IIHR-PSC1) and moderate sources of the aromatic amino acids (Phe, His, Tyr) in the bound fraction (IIHR-CA1, IIHR-AA1).

CONCLUSION

From the results of this study it can be concluded that the indigenous mushrooms collected from various parts of India, reveal a great diversity in their protein content (9.51-26.13%; highest in *P. cintrinopileatus* and *P. sajor-caju*), contents of free amino acids (11.56-73.11 mg Phe eq./ g DW; higher in *Hypsizygus tessulatus* and *Agrocybe aegerita*) and bound amino acids (57.41-171.85 mg Phe eq./ g DW; higher in *H. tessulatus*, *A. aegerita, H. ulmarius*, *P. djamor*, *P. florida*, *P. sajor-caju*), as also in the amino acid profiles. Of the essential amino acids, Ile

and Trp were significantly higher especially in the free fraction, comparable or higher than the best five plant sources. The free amino acid scores were higher in *H. erinaceus* (IIHR-HE1), *P. cystidiosus* (IIHR-PCYST1), *P. eryngi* (IIHR-PE2), *P. sajor-caju* (IIHR-PSC1); *H. tessulatus* (IIHR-BS1) and *A. aegerita* (IIHR-AA1) had higher bound amino acid content. Score of bound amino acids of isoleucine was higher, followed by the aromatic amino acids, phenylalanine and tyrosine. Thus, the mushrooms studied are an excellent source of Ile in the free fraction (IIHR-CA1, IIHR-AA1, ICAR-IIHR-HU1), good source of Trp (IIHR-HE1, IIHR-PCYST1, IIHR-PE2, IIHR-PSC1) and moderate sources of the aromatic amino acids (Phe, His, Tyr) in the bound fraction (IIHR-CA1, IIHR-AA1). Principal component analysis helped group the 18 mushroom isolates according to their free and bound essential amino acid content. The content of the conditionally essential and non-essential amino acids also revealed significant diversity. The data generated in this study has identified nutritionally superior mushroom isolates belonging to *H. erinaceus*, *P. cystidiosus*, *P. eryngi*, *P. sajor-caju, H. tessulatus* and *A. aegerita* by their amino acid profile, for commercialization.This can aid nutritionists and dieticians to formulate designer foods blending mushroom with vegetables/ grains/ millets for better health and nutrition, which can go a long way towards nutritional security.

Compliance with Ethical Standards: This article does not contain any studies with human participants or animals performed by any of the authors.

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(Supplementary data for amino acid profile)

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