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Nutritional and anti-nutritional components of some selected edible mushroom species

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The present investigation is carried on nutritional and anti-nutritional components in edible mushroom species of *Agaricus bisporus*, *Calocybe indica*, *Lentinula edodes, Macrocybe gigantea* (MA1), *M. gigantea* (MA2) and *Lentinus sajor-caju* syn. *Pleurotus sajor-caju*. Moisture content, total sugars, reducing sugars, protein content, ash content, extracellular enzymes (amylase, lipase and protease), minerals, tannins and phytic acid were assayed. All the species investigated contained considerable quantity of all nutritional and were low in anti-nutritional components. The values (mg/g) ranged from 38.66-47.73 for total carbohydrates, 9.02-17.77 for reducing sugars and 6.50-9.66 for total ash content. Protein content (g/100 g dry weight) ranged from 6.43 *in L. sajor-caju* to 26.2 in *L. edodes*. Among the minerals, phosphorus is the most abundant element in all mushroom species and it was maximum (944.5) in *M. gigantea* (MA2). The macronutrients (mg/100 g dry weight) in different mushroom species ranged between 345.8-944.5 (P), 21.8-68.8 (Ca) and 6.4-45.63 (Mg), while micronutrients ranged between 14.5-46.0 (Fe), 1.31-15.9 (Cu), 1.1-3.7 (Mn), 7.7-28.2 (Zn), 0.14-2.1 (Cr) and 0.004-0.39 (Co). None of the toxic elements like As, Hg or Pb was detected in any species. The anti-nutritional components like tannins (0.41-0.57 mg/g) and phytic acid (0.11-0.19 mg/g) were found below the threshold limits. The extracellular hydrolytic enzymes activity (mm) was maximum and minimum in *L. edodes* (34.1) and *A. bisporus* (9.16); *L. sajor-caju* (41.3) and *M. gigantea* (MA2) (22.3); and *C. indica* (90.0) and *A. bisporus* (34.6) for amylase, lipase and protease, respectively. The present study indicate that *A. bisporus* contain high nutrition (in terms of moisture content, total sugar, ash content, copper, zinc, iron and manganese) and low tannins and phytic acid content while *C. indica* contained least in all nutrition except protease activity.

Key words: Anti-nutrients, Edible mushrooms, Hydrolytic enzymes, Minerals, Nutrition, Phytic acid, Tannins.

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Introduction

Higher fungi have great potential to be used as food material[1] and the number of mushroom forming species[2] is estimated between 53,000 to 1,10,000. The sporocarps of edible fungus are richer in proteins than most of the vegetables, lower in fat content, rich in vitamins (Vitamin B, D, K and in some cases A and C is also reported) and minerals[3-7]. Trace amount of metals including iron, cobalt, manganese, copper, zinc and cadmium are necessary for living organisms, however, excess of these may become detrimental[8]. The intake of trace elements (essential or non-essential) above the threshold levels leads to morphological abnormalities including reduced growth and increased mortality. Average recommended intake for P, K, Ca, Fe, Cu, Mg, Mn, Zn and Na is 4000, 1000, 1000, 18, 2, 400, 2, 15 and 2400 mg/day, respectively[9]. Mushrooms have excellent ability to accumulate mineral elements from the growth medium[10,11].

Hydrolytic enzymes like amylase, lipase and protease catalyse larger molecules into smaller units[12]. Amylase hydrolyzes starch molecules and gives diverse products including dextrins and smaller polymers made up of glucose units[13]. Triglycerols are hydrolysed by lipases to glycerol and free fatty acids[14]. Lipases are associated with lipid metabolism including fat digestion, absorption, reconstitution and lipoprotein metabolism in eukaryotic organisms, whereas in plants they are found in energy reserve tissues[15]. Proteases are responsible for the breakdown of proteins into its building blocks namely amino acids[16].

In the present study, an attempt is made to examine the nutritional (total sugars, reducing sugars and proteins); minerals; anti-nutrients (phytic acid and tannins) and extracellular hydrolytic enzymes (amylase, lipase and protease) in six different edible mushroom species grown in the Tarai region of Uttarakhand, India.

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Materials and Methods

Sample preparation

Edible mushroom species of *Agaricus bisporus* (J.E. Lange) Imbach, *Calocybe indica* Purkay. & A. Chandra, *Lentinula edodes* (Berk.) Pegler, *Macrocybe gigantea* (Massee) Pegler & Lodge (MA1), *M. gigantea* (MA2) and *Lentinus sajor-caju* (Fr.) Fr. were selected and collected from Mushroom Research and Training Centre (MRTC), G.B. Pant University of Agriculture & Technology, Pantnagar. The fresh fruiting bodies of all species were washed thoroughly with tap water, then twice with distilled water to remove impurities and used for the estimation of total sugars and reducing sugars, whereas fruiting bodies were dried on blotting paper, kept in an oven at 65 ºC for 1 h and grounded to make fine powder for analysis of proteins, tanins and phytic acid. The mycelia of selected mushroom species/strains were cultured on medium for 10 days and zone of halo was measured for estimation of extracellular hydrolytic enzymes namely amylase, lipase and protease.

Moisture content determination

Five gram of fresh mushroom was weighed, dried in an oven at 50 °C, cooled in desiccator(s) and weighed in an electronic balance. The moisture content was calculated using the following formula[17]:

Moisture (%) =

(initial weight – final weight) × 100/weight of the sample

Total ash content

One gram of dried mushroom powder of each species was weighed and kept in a crucible then heated over low flame first for complete charring and then placed in muffle furnace (Ambassador temperature controller) for about 4-5 h at 600 °C. It was weighted after cooling in desiccator. To ensure ashing, the crucible was then heated in the muffle furnace for 1 h, cooled and weighed. This was repeated till two consecutive weights were same and ash was almost white or greyish colour. Total ash was calculated using following equation:

Ash content (g/100 g sample) =

weight of ash x 100/weight of sample

Extraction of sugars

Free sugars were extracted by using 1 g of fresh mushroom with 80 % ethanol and then 70 % ethanol (twice each) on boiling water bath for each species/strains. Ethanol was removed from pooled extract at 40 °C under diminished pressure to obtain concentrated aqueous syrup. The concentrated solution was transferred in volumetric flask and volume was made up to 98 mL with distilled water and 1 mL of saturated solution of basic lead acetate to precipitate proteins and a final volume of 100 mL with distilled water. The contents were filtered through Whatman No. 40 filter paper. The excess of lead ions were removed by treating with sodium oxalate, filtered off and used for estimation of free sugars[18].

Estimation of total sugars

Total sugars were estimated by the procedure given by Dubois *et al*[19]. One mL of test extract was mixed with 1 mL of 5 % phenol followed by 5 mL of concentrated H_2SO_4 and mixed well. After 10 min, the content was cooled under running tap water to room temperature. Absorbance was taken after another 20 min at 490 nm. Glucose standard curve (10-100 µg/mg) was plotted to calculate concentration of total sugars.

Reducing sugars

Reducing sugars were estimated by the method suggested by Nelson and Somogyi[20]. One mL of extract solution was added to 1 mL of copper tartrate reagent, incubated for 1 min in boiling water bath. One mL of arsenomolybdic acid reagent was added after cooling and the volume was made up to 5 mL with distilled water. A blue colour was developed and the intensity of the colour was measured at 620 nm by spectrophotometer (Tech Comp, UV 2300, Korea). The amount of reducing sugars was calculated from the standard curve of glucose.

Extraction and estimation of proteins

Mushroom powder (2 g) of each species was mixed with 10 mL of extraction buffer (Tris base 50 mM, EDTA (Na_2) 50 mM, triton X - 100 0.5 %, BME 0.3 %, ascorbic acid 0.39 %, PMSF 2 mM, PVP 0.4 %, pH 7.5) and cells were disrupted by pestle and mortar. The lysate was centrifuged at 12000 rpm for 30 min at 4 ºC and supernatant was used to estimate protein content. Estimation of protein concentration was calculated by the method suggested by Lowry *et al*[21]. Bovine serum albumin was taken as standard and absorbance was measured at 650 nm.

Estimation of anti-nutrients

Tannins

The tannin content was estimated by Folin-Ciocalteu reagent method as suggested by Attarde *et al*[22]. One mL each of extract and Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min. Then, 2.5 mL of 7.5 % sodium carbonate was added and further incubated for

30 min at room temperature. The absorbance was measured at 740 nm and the tannin content was expressed in terms of gallic acid equivalent (mg/g).

Phytic acid

Phytic acid was estimated by the method given by Wheeler and Ferrel[23]. About 10-40 mg of mushroom powder was extracted with 50 mL of 3 % of Tri chloro acetic acid (TCA) for 30 min and centrifuged. Then, 4 mL ferric chloride solution (2 mg ferric ions per mL in 3 % TCA) and 20 mL of 1.5 M KSCN was added and the developed colour was immediately measured at 480 nm against a reagent blank with each set of samples. Sodium phytate standard curve was used for calculating phytic acid content (mg/g) on dry weight basis.

Analysis of minerals

The phosphorus content was determined according to the method suggested by Sekine *et al* with modifications[24]. The method is based on the fact that when phosphorus reacts with molbdate vanadate reagent, it forms yellow coloured complex and estimated spectrophotometrically. The amount of phosphorus is calculated from the standard curve obtained by using 0.110 g KH_2PO_4/L and expressed as mg/100 g on dry weight basis. Ca, Fe, Mg, Cu, Cr, Co, Mn, Zn and As were estimated from ash content obtained from 1 g of dried mushroom after burning in muffle furnace at 600 ºC for 8 h, to which 2 mL of concentrated nitric acid was added and heated for 2 min. One drop of hydrogen peroxide was added to remove turbidity. The solution was transferred into a volumetric flask and total volume was made up to 50 mL by adding deionised water and used to analyse the content (mg/100 g) of Ca, Fe, Mg, Cu, Cr, Co, Mn, Zn and As by flame and graphite method with Atomic Absorption Spectroscopy (GBC scientific equipment sense AA dual serial no. A7240).

Extracellular enzymes

Amylase

For testing amylase activity, mycelia of different mushroom species were grown on glucose yeast extract peptone agar (GYP) medium (glucose 0.1 g, yeast extract 0.1 g, peptone 0.5 g, agar 16 g, distilled water 1000 mL) with 0.2 % soluble starch, pH 6.0. After incubation, the plates were flooded with 1 % iodine in 2 % potassium iodide[25].

Lipase

Lipase activity was assessed by growing the fungal mycelia of different species on peptone agar medium (peptone- 10 g, NaCl- 5 g, $CaCl_2H_2O$- 0.1 g, agar- 16 g, distilled water- 1 L; pH 6.0) supplemented with sterilized Tween 20. After incubation, visible precipitate is formed around the colony, due to the formation of calcium salts of the lauric acid liberated by the enzyme indicated positive lipase activity[25].

Protease

The proteolytic activity was assessed by growing the fungal mycelia of different species on sterilized GYP agar media (glucose 1 g, yeast extract 0.1 g, peptone 0.5 g, agar 16 g, distilled water 1000 mL, pH 6) with 0.4 % gelatine (pH 6.0) and to this, sterilized 8 % gelatin solution was added. After incubation, the culture was flooded with saturated aqueous ammonium sulphate. The clear zone around the colony indicates the hydrolysis of gelatin in media and the unhydrolysed gelatin is precipitated by ammonium sulphate[25].

Statistical analysis

All the experimental results were expressed as mean ± standard deviation of three replicates. The experimental data was subjected to analysis of variance for completely random design and the least significant difference was determined at the level of $P < 0.05$. Duncan's multiple range test was carried out using SPSS 16.0 for windows.

Results and Discussion

Nutritional analysis

Nutritional parameters (moisture content, total sugars, reducing sugars and protein) were studied in six different species of edible mushrooms (Table. 1). The moisture content was minimum (82.6 %) in *M. gigantea* (MA1) and maximum in *A. bisporus*

Table 1—Nutritional analysis in different edible mushroom species (mean±S.D.)

Species	Moisture content (%)	Total sugars (mg/g)	Reducing sugars (mg/g)	Ash content (mg/g)	Protein (g/100 g dw)
A. bisporus	90.9±0.39^{e}	47.7±0.46^{f}	16.0±0.34^{c}	9.66±0.05^{d}	14.0±1.30^{b}
C. indica	89.4±0.32^{d}	42.6±0.20^{d}	7.08±0.56^{a}	6.50±0.10^{b}	16.8±0.08^{c}
L. edodes	85.7±0.60^{b}	40.8±0.60^{c}	9.02±0.60^{b}	5.73±0.11^{a}	26.2±1.70^{d}
L. sajor-caju	88.7±0.02^{c}	39.5±0.70^{b}	15.5±0.64^{c}	6.43±0.32^{b}	6.43±0.60^{a}
M. gigantea (MA1)	82.6±0.05^{a}	38.6±0.30^{a}	8.41±0.79^{b}	6.23±0.05^{b}	16.4±0.36^{c}
M. gigantea (MA2)	89.2±0.04^{cd}	43.5±0.23^{e}	17.7±0.41^{d}	7.36±0.05^{c}	15.3±1.38^{bc}

* Values with different letters within columns indicate significant difference by Duncan's multiple range test ($P < 0.05$).

(90.9 %). The total sugars (mg/g) was maximum in *A. bisporus* (47.7), followed by *M. gigantea* (MA2) (43.5), *C. indica* (42.6), *L. edodes* (40.8), *L. sajor-caju* (39.5) and *M. gigantea* (MA1) (38.6), whereas reducing sugars (mg/g) was maximum in *M. gigantea* (MA2) (17.7), followed by *A. bisporus* (16.0), *L. sajor-caju* (15.5), *L. edodes* (9.02), *M. gigantea* (MA1) (8.41) and *C. indica* (7.08). The reducing sugar content in *M. gigantea* (MA2) is two and half times more than in *C. indica* (Table 1). The ash content was found to be the maximum in *A. bisporus,* followed by *M. gigantea* (MA2) and minimum in *L. edodes* (Table 1). Jeena *et al*[18] observed the ash and total sugar contents in different species of *Pleurotus* in the range of 13–148 mg/g and 28.85–37.30 mg/g, respectively. However, these values are comparatively much higher in ash content and lower in total sugar content than reported in the present study. Interestingly, *A. bisporus* exhibited higher content of moisture, total sugar and ash, followed by *M. gigantea* (MA2) compared to other species.

Protein content

The protein content (g/100 g dry weight) ranged from 6.43 in *L. sajor-caju* to 26.2 in *L. edodes* (Table 1). Alam *et al*[26] reported a higher range (20.56–24.63 g/ 100 g) of proteins in different species of the genus *Pleurotus* and *C. indica*. These values are higher than the present findings. Poongkodi *et al*[27] reported protein content in the pilus and stipe of *A. bisporus* (32.0 and 28.98 g/100 g) and *P. ostreatus* (12.99 and 11.20 g/100 g) and these values are higher than the values in the present study.

Mineral elements profile

The mineral content (mg/100 g) observed in different mushroom species studied is given in Table 2. The P content (mg/100 g) was maximum in *M. gigantea* (MA2) (944.5), followed by *M. gigantea* (MA1) (601.5), two different strains of *M. gigantea*[28] and lowest in *C. indica* (56.36). The rest of minerals in different mushroom species ranged in between 1.3-15.9 (Cu), 7.70-28.2 (Zn), 0.10-2.13 (Cr), 14.5-46.1 (Fe), 21.8-68.8 (Ca), 1.1-3.7 (Mn), 6.4-45.6 (Mg) and 0.03-0.39 (Co). Among the species studied, maximum content (mg/100 g) of Cu (15.9), Zn (28.2), Fe (46.1) and Mn (3.7) was found in *A. bisporus*; Ca (68.8) and Mg (45.6) in *L. edodes*; Cr (2.13) in *L. sajor-caju*; Co (0.39) in *M. gigantea* (MA1) and P (944.5) in *M. gigantea* (MA2). Interestingly, *C. indica* contained least amount in six out of nine elements, while *L. edodes*, *M. gigantea* (MA2) and *M. gigantea* (MA1) contained least amount of Zn, Fe and Cr, respectively (Table 2). The maximum amount obtained in *A. bisporus* was approximately twelve and three times higher than *C. indica* in respect of Cu and Mn, respectively*;* more than three times higher than *L. edodes* in respect of Zn*,* three times higher than *M. gigantea* (MA2) in respect of Fe. Genccelep *et al*[29] determined mineral content in wild grown edible mushroom species and reported 1.12-4.49 mg/g of P; 0.17–8.80 mg/g of Ca; 0.90–9.71 mg/g of Mg; and 50.3–842.0 mg/ Kg of Fe and these values are comparatively lower than the present study values except Fe, which is more or less equal. Sesli and Tuzen[30] reported comparatively lower values of Fe (31.3–1190 µg/g) and Cu (10.3–145 µg/g) content in different mushroom species. Similarly, Tuzen[31] reported, comparatively lower values (12-181 µg/g) in different mushroom species. The manganese content in earlier studies[32-34] have been reported in the range of 7.1–81.3 µg/g, 14.2–69.7 µg/g and 21.7–74.3 µg/g

Table 2—Mineral content (mg/100 g) in different edible mushroom species (mean±S.D.)

Minerals	*A. bisporus*	*C. indica*	*L. edodes*	*L. sajor-caju*	*M. gigantea* (MA1)	*M. gigantea* (MA2)
P	345.8±0.15^{a}	56.36±0.32^{d}	465.4±0.51^{c}	412.0±0.45^{b}	601.5±0.30^{e}	944.5±0.10^{f}
Cu	15.9±0.15^{d}	1.3±0.01^{a}	1.41±0.005^{a}	9.52±0.35^{c}	2.08±0.07^{b}	2.24±0.13^{b}
Zn	28.2±0.32^{f}	9.6±0.1^{b}	7.70±0.2^{a}	18.1±0.15^{e}	13.3± 0.20^{c}	14.5±0.41^{d}
Cr	1.2±0.01^{c}	0.1±0.005^{a}	0.14±0.01^{a}	2.13±0.05^{d}	0.24±0.04^{b}	0.24±0.02^{b}
Fe	46.1±0.10^{f}	19.2±0.32^{b}	19.7±0.11^{d}	27.0±0.02^{e}	17.6±0.43^{b}	14.5±0.26^{a}
Ca	39.5±0.40^{e}	21.8±0.1^{a}	68.8±0.05^{f}	24.0±0.02^{b}	26.4±0.10^{c}	27.2±0.09^{d}
Mn	3.7±0.15^{d}	1.1±0.01^{a}	3.06±0.05^{c}	3.16±0.11^{c}	2.23±0.15^{b}	2.33±0.15^{b}
Mg	28.2±0.40^{e}	6.4±0.05^{a}	45.6±0.35^{f}	19.5±0.11^{d}	10.7±0.26^{b}	15.73±0.28^{c}
Co	0.03±0.28^{a}	0.03±0.02^{a}	0.04±0.01^{a}	0.004±0.001^{a}	0.39±0.08^{c}	0.26±0.07^{b}
As	-	-	-	-	-	-
Pb	-	-	-	-	-	-
Hg	-	-	-	-	-	-

* Values with different letters within columns indicate significant difference by Duncan's multiple range test ($P < 0.05$); **- -** not detected

and these values are comparatively lower than the present results (Table 2). In the present study, none of the toxic elements like lead, arsenic or mercury were detected in any of the species. Agrahar-Murugkar and Subbulakshmi[35] have studied mineral composition of *Calvatia gigantean*, *Cantharellus cibarius*, *Russula integra*, *Gomphus floccosus* and *Lactarius quieticolor* and reported higher content than reported in the present study.

Anti-nutrients analysis

Anti-nutrients (tannins and phytic acid) were examined and the results are presented in Table 3. The tannin content ranged from 0.41±0.03 in *A. bisporus* to 0.57±0.01 mg/g in *C. indica*. The tannin content were within standard of safe limit (60.00 mg/100 g)[36]. The phytic acid content (mg/g) was minimum in *A. bisporus* (0.11) and maximum in both *M. gigantea* (MA1) and *C. indica* (0.19). The amount of phytic acid in *A. bisporus* is approximately half compared to both in *M. gigantea* (MA1) and *C. indica.* Jeena *et al*[18] also reported similar values for tannins and phytic acid in *L. sajor-caju*, *P. ostreatus* and *P. sapindus*. The values of phytic acid content in different mushroom species in the present study are over 100 times lower than the standard safe limit (22.10 mg/100 g)[36], indicating that the studied species are highly safe with respect to toxicities associated with phytate concentration. On the basis of anti-nutrient content (tannins and phytic acid), *A. bisporus* is comparatively the best among the selected mushroom species.

Extracellular enzymes

The activity of hydrolytic enzymes (amylase, lipase and protease) was measured by halo zone size of clearance (mm) after 10 days of incubation. Protease activity was highest among the hydrolytic enzymes in all the species (Table 4). The amylase activity ranged from 9.16 mm in *A. bisporus* to 34.1 mm in *L. edodes*, indicating that *L. edodes* is better producer of amylase among the selected species. Lipase activity ranged from 22.3 mm in *M. gigantea* (MA2) to 41.3 mm in *L. sajor-caju*. Thus, *L. sajor-caju* showed stronger whereas *M. gigantea* (MA2) showed weaker lipase activity among the species studied. The clearance zone of halos for protease ranged from 34.6 mm in *A. bisporus* to 90.0 mm in *C. indica*. Goud *et al*[37] examined the production of extracellular enzymes in 50 South Indian basidiomycetes species and found amylase activity in the range of 5–28 mm, lipase 22–43 mm and protease 13–45 mm. These values indicate lower activity of both amylase and protease while lipase activity is more or less similar to the present study (Table 4).

Table 3—Antinutrients (mg/g) in different edible mushroom species (mean±S.D.)

Species	Tanins	Phytic acid
A. bisporus	0.41±0.03^{a}	0.11±0.01^{a}
C. indica	0.57±0.01^{d}	0.19±0.01^{c}
L. edodes	0.43±0.02^{ab}	0.15±0.01^{b}
L. sajor-caju	0.47±0.01^{bc}	0.18±0.03^{bc}
M. gigantea (MA1)	0.52±0.01^{c}	0.19±0.01^{c}
M. gigantea (MA2)	0.44±0.03^{ab}	0.17±0.02^{bc}

* Values with different letters within columns indicate significant difference by Duncan's multiple range test ($P < 0.05$).

Table 4—Extracellular hydrolytic enzymes (mm) in different edible mushroom species (mean±S.D.)

Species	Amylase (mm)	Lipase (mm)	Protease (mm)
A. bisporus	9.16±0.28^{a}	31.3±1.52^{c}	34.6±1.15^{b}
C. indica	15.0±1.00^{c}	26.0±2.00^{b}	90.0±1.00^{a}
L. edodes	34.1±1.89^{e}	33.6±1.52^{c}	50.6±2.08^{d}
L. sajor-caju	20.5±0.50^{d}	41.3±1.52^{d}	72.6±2.51^{e}
M. gigantea (MA1)	21.0±1.00^{d}	25.3±0.57^{b}	52.0±1.00^{d}
M. gigantea (MA2)	13.0±1.00^{b}	22.3±1.15^{a}	45.6±1.52^{c}

* Values with different letters within columns indicate significant difference by Duncan's multiple range test ($P < 0.05$).

Conclusion

The results indicate the presence of substantial amount of sugars, proteins, minerals and extracellular enzymes in all the six selected edible mushroom species. Further, the content of anti-nutrients (both tannins and phytic acid) was below the threshold limits in all the species. The findings also suggest that *A. bisporus* with highest moisture, total sugars, copper, zinc, iron and manganese content and lowest content of anti-nutrients is the best among the selected mushroom species. *L. edodes* was found with maximum amount of protein. Considering human health, these species of mushroom may be source of potential food. Further, toxic elements were not detected in any of the selected mushrooms. On one hand mushroom contains high nutritive potential and on the other hand produces hydrolytic enzymes thus helpful in digestion. Therefore, the above selected mushrooms not only have nutritive potential but also self produces hydrolytic enzymes for digestion and hence can be considered as good source of nutraceuticals.

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