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Comparative Biochemical and Biophysical Evaluation of Ecofriendly Mushrooms of Rajasthan

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Abstract

Fruiting body of six different mushrooms namely, *Agaricus bisporus*, *Hyspizygus ulmarius*, *Pleurotus florida* PF-01, *Pleurotus florida* PF-01 R_5, *Pleurotus platypus* and *Pleurotus sajor-caju* PSC-04 were taken from Udaipur and analyzed for their comparative biochemical and biophysical properties. The maximum soluble protein was observed in *P. florida* PF-01 R_5 (5.56 mg /g fresh weight) while minimum in *H. ulmarius* (2.70 mg/g fresh weight), maximum reducing sugar was observed in *H. ulmarius* (18.22 mg/g fresh weight), while minimum in *P. platypus* (13.06 mg/g fresh weight). Comparison on the basis of biochemical and biophysical characteristics showed that cultivated strains of mushrooms (viz. *A. bisporus*) have low enzyme activities while wild ones (*P. platypus*, *P. sajor-caju* PSC-04 and *H. ulmarius)* have high enzyme activities. Thus, if wild counterparts of mushrooms are used for food purposes, more nutrients and antioxidants can be taken through diet.

Key words : Mushrooms, Proximate composition, Soluble protein, Reducing sugar, Superoxide dismutase.

World production of cultivated edible mushroom has been increased from 170 metric tons (MT) in 1960 to 7,000 × 10^3 MT in 1997-98. China has become a giant producer and consumer of mushrooms and a large share (70%) of the total world production come from China (Chang and Miles 2004). India's share is estimated to be around 70,000 MT per annum in which major share (85%) is contributed by button mushroom (*Agaricus* spp.) (Singh et al. 2003). Udaipur, Jaipur, Ajmer, Bikaner, Bhilwara, Kota, Bundi, Jalore, Jodhpur, Sikar, Sriganganagar, Banswara, Tonk and Pali are major mushroom growing districts in Rajasthan. The state produced 200 tons of mushrooms during 1997-98 (Kumar et al. 2007).

Diversified agro-climatic conditions in India offer vast potential for growing different types of mushrooms. There are about 20 varieties of mushrooms being cultivated throughout the world as food. In India, only three, viz. white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.) and paddy straw mushroom (*Volvariella volvacea*) are grown commercially. Out of these, white button mushroom contribute about 90% of the total production. Mushrooms are rich source of proteins, minerals and vitamins. They have been recognized as the alternate source of good quality protein and producing the highest quantity of protein per unit area from the agro-wastes. Besides, they provide potentiality for generating employment, improving economic status of growers, help in checking pollution and as tool for earning foreign currency (Rai and Arumuganathan, 2005). Mushrooms contain nearly 90% moisture and are highly perishable in nature. They keep respiring after harvest and many changes like browning, liquefaction and loss of moisture and texture occur, resulting in reduced market value and acceptability. At ambient conditions mushrooms have a short shelf life of 1—2 days. Further, as diet consciousness is increasing in this era, mushrooms are considered an important health food and thus, find a place in recommended diet of patients. In a developing and overpopulated country like India, the problem of malnutrition is quite obvious. At present it has almost the lowest rate of protein consumption in the world which is 5 g/day

Table 1. Proximate composition and biophysical characteristics of different accessions of mushroom. Em = Energy derived by moisture equation of Bradbury (1986).

Strains	Moisture (%)	Dry matter (%)	Em (kcal)	Re-hydration ratio
Agaricus bisporus	92.00	8.00	23.90	1.21
Hyspizygus ulmarius	89.50	10.50	34.28	2.13
Pleurotus florida PF-01	90.50	9.50	30.12	1.63
Pleurotus florida PF-01 R_5	85.00	15.00	52.96	2.58
Pleurotus platypus	86.00	14.00	48.81	1.87
Pleurotus sajor-caju PSC-04	91.00	9.00	28.05	2.33

Table 2. Biochemical characteristics of different accessions of mushroom.

Strains	Soluble protein (mg/g of fresh weight)	Reducing sugar (mg/g of fresh weight)	SOD activity (units/mg protein)	POX activity (units/mg protein)
Agaricus bisporus	5.30	16.66	1.69	317
Hyspizygus ulmarius	2.70	18.22	12.27	622
Pleurotus florida PF-01	5.33	15.14	8.33	360
Pleurotus florida PF-01 R_5	5.56	15.06	2.55	777
Pleurotus platypus	3.86	13.06	2.68	1990
Pleurotus sajor-caju PSC-04	4.20	14.74	19.06	257
SE ±	0.135	0.216	0.0194	8.01
CD (*P* = 0.05)	0.415	0.665	0.0598	24.68

per person (Rai et al. 2005). Many edible mushrooms are reported and thousands of unidentified strains are present especially in Aravali region of Rajasthan. Fruiting bodies of wild and cultivated six strains of mushrooms were obtained fresh just after harvesting from All India Co-ordinated Mushroom Improvement Project (AICMIP), Udaipur and analyzed for their biochemical and biophysical properties to explore their nutrient and antioxidant status.

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Methods

Six different mushrooms viz. *Agaricus bisporus, Hyspizygus ulmarius, Pleurotus florida* PF-01, *Pleurotus florida* PF-01 R_5, *Pleurotus platypus* and *Pleurotus sajor-caju* PSC-04 were taken from All India Co-ordinated Mushroom Improvement Project, Department of Plant Pathology, Rajasthan College of Agriculture, MPUAT, Udaipur. Fruiting bodies of wild and cultivated strains of mushrooms were obtained fresh just after harvesting, cleaned, washed in running tap water and analyzed for their comparative biochemical and biophysical properties.

Moisture and Dry Matter Percent

Moisture content was determined by oven drying method (Ranganna, 2000), while dry matter per cent was calculated as follows (Sawhney and Singh, 2002):

Dry matter per cent = 100 – moisture per cent

Calorific Value

Calorific value was calculated using the moisture based equation of Bradbury (1986).

$$E_m = -1699 + 17.38\,M$$

Where, E_m = Calorific value obtained by moisture data, M = Moisture per cent (%) of a strain in question. The resulting value was divided by factor of 4.186 to obtain energy value in Kcal.

Re-Hydration Ratio (RR)

For re-hydration ratio, slightly modified method of Kumar et al. (2007) was followed. Pre-weighed samples were soaked in ample amount of water for 30 min at room temperature. The ratio of mass of re-hydrated and normal samples was used to find the re-hydration ratio :

Re-hydration ratio = Weight of re-hydrated sample (g)/Test weight of sample (g)

Preparation of Crude Extract and Biochemical Estimations

After preliminary studies of standardizations with respect to buffer, pH, extraction conditions, the extractions were done following the procedure : 5.0 g of fresh tissue (mushroom fruiting body) was homogenized with 10.0 ml of 0.1 M tris-HCl buffer (pH 7.5) containing 1 mM EDTA, 3% polyvinyl pyrrolidone and 1 mM $CaCl_2$ in a pre-chilled pestle and mortar using acid washed sand as an abrasive. The homogenate was filtered through four layers of cheesecloth and the filtrate centrifuged at 12,000 rpm (14,166 g) for 15 min in a refrigerated centrifuge (Remi, India) at 4C. The supernatant obtained was used as crude preparation for enzyme assays and other biochemical estimations. Soluble protein was estimated as described by Bradford (1976) using bovine serum albumin as standard while reducing sugars were estimated using dinitrosalicylic acid reagent adopting the method of Miller (1959). Both parameters are expressed in mg/g fresh weight of mushrooms.

Assay of Superoxide Dismutase Activity

Superoxide dismutase was assayed by measuring its ability to inhibit the photo-chemical reduction of nitroblue tetrazolium (NBT) adopting the method of Beauchamp and Fridovich (1971). The reaction mixture 3.0 ml contained 0.05 M Tris-HCl (pH 7.8), 14 mM L-methionine, 60 µM NBT, 3 µM riboflavin, 0.1 mM EDTA and 0.2 ml of enzyme extract. Riboflavin was added at the end. The tubes were shaken and placed 30 cm below light source consisting of two 100W fluorescent lamps (Phillips, India). The reaction was started by switching on the light and terminated after 20 min of incubation by switching off the light. After terminating the reaction, the tubes were covered with black cloth to protect them from light. A non-irradiated reaction mixture that did not develop color served as the control. The reaction mixture developed maximum color without enzyme extract and its absorbance decreased with the addition of enzyme. The absorbance was recorded by spectrophotometer (Elico, India) at 560 nm. Per cent inhibition was calculated by the following formula of Asada et al. (1974).

$$\text{Per cent inhibition} = \frac{V-v}{V} \times 100$$

Where, V =Rate of assay reaction in absence of SOD, v= Rate of assay reaction in presence of SOD.

One enzyme unit is defined as the amount of enzyme, which could cause 50% inhibition of the photochemical reduction of NBT (McCord and Fridovich 1969). However, for the purpose of kinetic and regulatory properties, the enzyme activity was expressed in terms of units/ml and calculated by the commonly used formula of Giannopolitis and Ries (1977). Finally activity was expressed units/mg protein.

$$\text{SOD units/ml} = \frac{V-v}{v}$$

Assay of Peroxidase Activity

Peroxidase activity was assayed by the modified method of Seevers et al. (1971). The reaction mixture contained 0.1 ml of 2.0% O–dianisidine in methanol, 0.1 ml of 10 mM H_2O_2, 2.7 ml of 0.1 M sodium acetate buffer (pH 5.0) and properly diluted aliquots of enzyme extract (0.1 ml). The reaction was initiated by the addition of H_2O_2 and change in absorbance was followed at 470 nm spectrophotometrically (Systronics, India) upto 3 min. The linear change in absorbance was taken to calculate enzyme activity. One unit of enzyme activity was defined as 0.001 change in OD per min (Neves 2002). Finally activity was expressed as units/mg protein.

Results and Discussion

The proximate compositions of different accessions of mushroom are presented in Table 1. The moisture content of six varieties of mushrooms ranged from 85 to 92%. The highest moisture content was observed in *A. bisporus,* (92.0%) whereas the lowest moisture content was observed in *P. florida* PF-01 R_5, (85.0%). Conversely, the highest dry matter was observed in *P. florida* PF-01 R_5 (15.0%), whereas the lowest solid content was observed in *A. bisporus* (8.0%). Moisture based calorific value was also enumerated, and it was found that all mushroom accessions have low calorific values compared to other food commodities. General range of calorific value was 23.90 kcal (*A. bisporus*) to 52.96 kcal (*P. florida* PF-01 R_5). Mushrooms are highly perishable foods having moisture content upto 85—90%, the rest being dry matter. They have negligible fat and less calorific values (Rai et al. 2005). Similar results have been reported by Rai and Arumuganathan (2005), Gopalan et al. (2002) and Kumar et al. (2007). Rai and Arumuganathan (2005) gave the proximate composition of *A. bisporus, P. sajor-caju* and *P. florida.* Moisture content was nearly 90% in all the strains. Dry matter content ranged from 6—10% and calorific value ranged from 16 to 36 kcal. Kumar et al. (2007) while investigating proximate composition of *A. bisporus* and *P. sajor-caju* reported that the former contained 88.95% moisture, 11.05% dry matter and 33.0 kcal of energy while the latter had 87.4% moisture, 12.6% dry matter. Our results are in conformity with that of the Rai and Arumuganathan (2005), Gopalan et al. (2002) and Kumar et al. (2007). Under biophysical attributes, re-hydration ratio (Table 1) was minimum in *A. bisporus* (1.21) while maximum in *P. florida* PF 01 R_5 (2.58). Kumar et al. (2007) reported rehydration ratio in dehydrated *A. bisporus* nearly 2.3 while in dehydrated *P. sajor-caju* 3.8. Singh et al. (2007) studied post drying characteristics of button mushroom. The rehydration ratio was found in the range of 1.90 to 2.49.

Soluble protein was estimated to calculate specific activities of SOD and POX activities (Table 2). The maximum soluble protein was observed in *P. florida* PF-01 R_5 (5.56 mg/g fresh weight) while minimum in *H. ulmarius* (2.70 mg/g fresh weight). On the other hand, reducing sugars ranged from a minimum of 36.06 mg/g on fresh weight basis in *P. platypus* to a maximum of 18.22 mg/g on fresh weight basis in case of *H. ulmarius* (Table 2).

SOD activity was expressed in units/mg protein and it ranged from a minimum of 1.69 units/mg protein in *A. bisporus* to 19.06 units/mg protein in *P. sajor-caju* PSC-04 (Table 2). While activity of POX ranged from a minimum of 317 units/mg protein in *A. bisporus* to a maximum of 1990 units/mg protein in *P. playtypus.* However, Flurkey et al. (1994). investigated that peroxidase activity was low or undetectable in *Agaricus,* oyster and shiitake mushrooms. Control of enzymatic browning in different mushroom types may depend upon the distribution of different oxidases within any given type. Nayyar and Kaushal (2002) also reported that the increased activity of catalase and peroxidase enzyme constitutes potential defense mechanism against chilling induced oxidative damage in germinating wheat grains.

Conclusion

Comparison on the basis of biochemical and biophysical characteristics, especially, on the basis of soluble protein, SOD and POX activities show that cultivated strains of mushrooms (viz. *A. bisporus*) have low enzyme activities while wild ones (*viz. P. platypus, P. sajor-caju PSC-04 and H. ulmarius*) have high enzyme activities. Thus, high nutrients and antioxidants can be taken if wild counterparts of mushrooms used for food purposes.

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