Total soluble protein, phenols and nitrogen contents of *Pleurotus* djamor (Fr.) Boedijn

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Analyses from different stages of white ecological variant of pan tropical mushroom *Pleurotus djamor* (Fr.) Boedijn procured from northern tropical moist deciduous forests of Tripura (India) revealed that it contained 0.12%-0.27% of total phenols, 1.88 – 2.96% of total soluble protein and 0.10-0.44% total nitrogen on the fresh weight basis. Both total soluble protein and total nitrogen parameters were found to be highest in juvenile bud stage and gradually decreased in consecutive other stages i.e., pre-mature (two days old), mature (three days old) and post-mature (four days old) stages. Besides, quantitative estimation of total phenols revealed that the species showed highest amount of phenols in juvenile bud stage (one day stage) and gradually decreased, but at post-mature (four days old) condition, amount of total phenol again increased. From these studies, it was concluded that the consumption of this edible mushroom would be better at 2-3 days mature stage but not at juvenile stage or post-mature (four days old) stage.

Key words: Pleurotus djamor (Fr.) Boedijn., soluble protein, total nitrogen, total phenols

INTRODUCTION

Perhaps no subject is of more interest to the people in the day-to-day life than the search for palatable foodstuffs and determination of their dietary constituents. Everyone needs to secure one's life with foodstuffs which should be contributed with better flavour and taste, better source of protein, having acceptable biting properties, low calorie as well as cost effective, easily digestible and should be nontoxic (Alvarez-Parrilla et al., 2007). Mushrooms represent one of the world's greatest untapped resources of nutritious and palatable foods. Mushrooms have been used as traditional foods and medicines in different parts of the world, including Asia, Africa and America (Alvarez-Parrilla et al., 2007). As in America and other corners of the world, wild edible mushrooms have been a part of the diet, especially among ethnic groups (Alvarez-Parrilla et al., 2007). They have nutritional relevance due to their high fiber, minerals and protein content, as well as low fat content (León-Guzman et al.,

1997). Several studies analyzing the total soluble total phenols, total amino compositions and antioxidant activity of fresh and cooked wild and commercial mushrooms have been published (Mau et al., 2001, Lakshmi et al., 2004; Lo and Cheung, 2005, Choi et al., 2006, Ferreira et al., 2007, Alvarez-Parrilla et al., 2007). Among the antioxidant compounds, polyphenols have gained importance due to their large array of biological actions that include free radical scavenging, metal chelation and enzyme modulation activities, inhibition of low density lipoprotein (LDL) oxidation. (Rodrigo and Bosco, 2006; Alvarex-Parrilla et al., 2007). The term 'polyphenol' refers to a complex group of compounds that includes in their structure an aromatic ring bearing one or more hydroxyl groups. They include simple phenols such as phenolic acids and derivatives, as well as complex such as flavones, flavonoids, anthocyanins, among others (Alvarez-Parrilla et al., 2007). The objective of this study has been to analyze the differences among various stages of wild *Pleurotus djamor* mushroom in accordance with their level of total soluble protein, total nitrogen and total phenols collected from the mixed type of forest of Tripura.

MATERIALS AND METHODS

Collection of samples

Wild mushrooms of various stages were collected at the end of the rainy season from northern tropical moist deciduous forests of Tripura (20°51'-24°32' N latitude and 90°10′-92°21′ E longitude) during July-Ausust, 2010. Ten to twenty mushrooms of each stages (10 g) were collected and kept on ice 4-6 h for transportation to the laboratory. Mushrooms were classified up to species level, using morphological and anatomical characteristics. Mushrooms samples were sent to Mycological Research Laboratory of ICAR, Lembucherra, for identification and were identified as Pleurotus djamor (Fr.) Boedijn. (white ecological variety). Wild mushrooms were cut, weighted and frozen at -10°C for 1 day, lyophilized for 48 h (Labconco Freeze dry/shell freeze system), milled and stored at -10°C. In order to minimize variability between individuals from the same species, all mushrooms from the same specie were homogenized according to the modified method of Alvarez-Parrilla et al. (2007). Different stages of mushroom were expressed in the following terms juvenile (one day old), pre-mature (two days old), mature (three days old) and post-mature (four days old)

Proximate analysis of total (phosphate buffer) soluble protein

The total (phosphate buffer) soluble protein was estimated from the stipe connecting pileus of mushroom for different stages (24, 48, 72 and 96 h) after soaking treatment. The procedure of Lowry et al. (1951) was followed for estimation of total (phosphate buffer) soluble protein. The standard curve for protein was prepared using boving serum albumin (BSA) as standard protein. The unknown amount of protein in the sample was determined using a standard curve and also taking into account all the possible variables. Amount of total soluble protein was estimated as g per 100 g fresh weight (FW) basis.

Proximate analysis of total nitrogen

The level of total nitrogen (soluble and insoluble) were estimated by the modified Micro-Kjeldahl method of Lang (1958). The ethanol soluble and insoluble nitrogen fractions were estimated at different stages in oven dried samples of stipe connecting pileus part of mushroom. Amount of total nitrogen was estimated as g per 100 g fresh weight (FW) basis.

Determination of total phenols

Mushroom extracts were obtained according to the methodology proposed by Kähkönen et al. (1999) and Alvarez-Parrilla et al. (2007). Total phenols were determined according to the method reported by Georgé et al. (2005), with the Folin-Ciocalteu reagent, using tannic acid in ethanol (80%) as standard. Absorbance at 650 nm was determined by using Systronics UV-VIS Model-117 Spectrophotometer and results were expressed as mg of tannic acid (TNA) per 100 g fresh weight (FW) basis.

Statistical analysis

Values are presented as the mean ± SEm of five replicates. For standard error of the mean (SEm), the obtained results (data) were statistically analyzed according to Steel and Torrie (1980). Two-way ANOVA analyses were performed in order to determine differences between various biochemical parameters and various stages of mushrooms at 5% significant level, using the commercial software SPSS 13.0 (SPSS Inc. Headquarters Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Mushrooms apart from being famous for their appetizing flavour, offer themselves as potential protein source. The great advantage is that mushrooms have the capacity to convert nutritionally valueless substances into high protein food (Chang and Hayes, 1978). The digestibility of mushroom protein was as high as 72-83% (Lintzel, 1941). Mushrooms are useful for diabetic and heart patients and an important source of nutritive proteins and minerals to the vegetarians (Bahl, 1994). Initially the

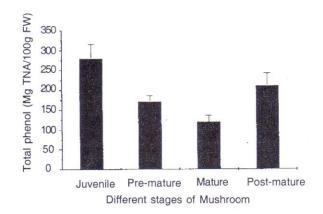


Fig 1. Amount of total phenols on stipe connecting pileus part in different stages of mushroom

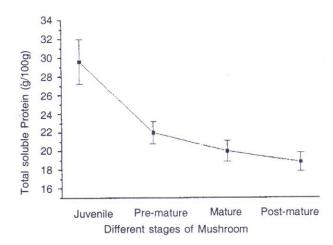


Fig 2. Amount of total soluble protein in different stages of mushroom

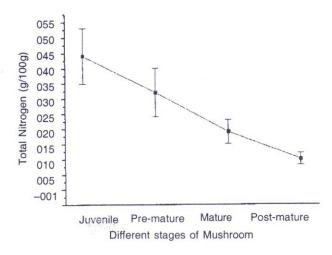


Fig 3. Amount of total nitrogen in different stages of mushroom

crude protein content of the common mushroom *Agaricus bisporus* has been reported to be 19-38% on a dry weight (DW) basis.

The present studied mushroom is under the genus Pleurotus. More than 100 strains of Pleurotus have been cultivated (Mata and Salmones, 2003), mainly strains of P. ostreatus (Jacq. : Fr.) Kumm., P. columbinus Bres., P. pulmonarius (Fr.) Quel., and P. djamor (Fr.) Boedijn (pink and white ecological varieties). The present mushroom under the investigation is a white ecological variety of P. djamor (Fr.) Boedijn. The data of Table 1 revealed that the total soluble protein content was highest (2.96 g/100 g fresh weight) at juvenile stage (1 day) and gradually decreased in pre-mature, mature and post mature stages (Fig. 2). Same trends were found for total nitrogen content which was found highest (0.44 g/100 g fresh weight) at juvenile stage (1 day) and gradually decreased in pre-mature, mature and post mature stages (Fig. 3).

In literature, protein analysis of Pleurotus ostreatus was found to be 15-35% and 4.6-33% on fresh and dry weight basis, respectively (Chang and Hayes, 1978; Hafiz et al., 2003). Mushrooms, the fruiting bodies of fungi, are appreciated not only for their texture and flavour but also for their high protein and other nutritional characteristics (Manzi et al., 1999). Nutritionally, mushrooms provide key nutrients and bioactive components such as high-quality proteins, vitamins, minerals, unsaturated fatty acids and fibers (Danell and Eaker, 1992; Buswell and Chang, 1993; Omarini et al., 2010). P. tuber-regium, contained crude protein ranging from 4.1 to 13.8% with the highest concentration in the cap (13.8%) than other parts. In P. eryngii, the level of protein was reported to be 24.08% on fresh weight basis (Hassan et al., 2010). But varying amount of protein contents in edible mushrooms from other parts of the world were also reported. For example, Adejumo and Awosanya (2005) reported protein contents of two mushrooms i.e., Auricularia sp. (with 4-9% protein) and Agaricus species (with 24-44% protein) from Africa, whereas Kalyoncu et al., (2010) reported higher amount of protein in three edible mushrooms Sparassis crispa (83.40%), Armillaria mellea (76.94%) and Meripilus giganteus (75.56%) from Antolia. Pleurotus sajor-caju fruit bodies when grown in three different substrates, showed varied level of proteins content - highest (16%) in wheat straw,

Table 1: Estimation of total protein, phenols and nitrogen in stipe connecting pileus part of Pleurotus djamor

Mushroom sample	Amount of biochemical parameters				
	Total phenol	Total protein	Total nitrogen		
	(mg/100g)	(g/100 g)	(g/100 g)		
Juvenile (1 day stage)	279.119 ± 0.042*	2.960 ± 0.031	0.440 ± 0.061		
Pre-mature (2 days stage)	144.754 ± 0.065	2.211 ± 0.021	0.334 ± 0.054		
Mature (3 days stage)	127.105 ± 0.018	1.986 ± 0.032	0.210 ± 0.010		
Post-mature (4 days stage)	208.544 ± 0.021	1.880 ± 0.051	0.100 ± 0.007		

^{*}All the readings are based on 5 replicates ± SEM

Table 2: Two-Way ANOVA analyses of the data

Sources of variation	df	SS	MS .	F	F(0.05)
Row	4	0.02251	0.00752	4.268	3.0069
Column	4	0.96582	0.04112	26.635	3.0069
Error	16	0.01478	0.00215		
Total	24	1.00311			

medium (13.4%) in water hyacinth and lowest (10.8%) in corn cobs substrate (Kihumbu *et al.*, 2008).

Though available literature of biochemical parameters for *P. djamor* is very meager, the present result is in accordance with the previous results for edible pileus parts of the different species of *Pleurotus* as well as for other genera also and agreed the significant level of protein and nitrogen contents (Petrovska. 2001; Oyetayo and Oyetayo, 2005; Khan *et al.*, 2009; Manjunathan and Kaviyarasan, 2010; Cuptapun *et al.*, 2010). The variation may be due to the variation in species of mushrooms or due to the stages of development or due to variation in soil and location (Flegg and Maw, 1977).

The data from Table 1 revealed that the total phenol content was highest (279.119 mg/100 g fresh weight) at juvenile stage and gradually decreased in pre-mature and mature stage. But it increased up to 208.544 mg/100 g FW in post mature stage (Fig. 1). Mushroom contains various polyphenolic compounds recognized as an excellent antioxidant due to their ability to scavenge free radicals by single electron transfer

(Hirano *et al.*, 2001). Some common edible mushrooms which are widely consumed in Asian countries, have currently been found to possess antioxidant activity which also correlated with their total phenolic content (Cheung and Cheung, 2005; Cheung *et al.*, 2003; Lo and Cheung, 2005; Mau *et al.*, 2004; Yang *et al.*, 2002; Yen and Hung, 2000).

High phenolic compounds might account for good antioxidant properties found in all species of Leucopaxillus giganteus (629 mg/100 g), Sarcodon imbricatus (376 mg/100 g) and Agaricus arvensis (283 mg/ 100 g) (Barros et al., 2007). Among the antioxidant compounds, polyphenols have gained importance due to their large array of biological actions that include free radical scavenging, metal chelation, enzyme modulation activities and inhibition of LDL oxidation (Rodrigo and Bosco, 2006; Alvarez-Parrilla et al., 2007).

Statistical analyses (Table 2) considering the amount of chemical constituents in each experimental set (two-way ANOVA) revealed that calculated F (26.635) for various amount of biochemical parameters in stipe connecting pileus part of the present studied mushroom was much

higher than tabulated F (3.0069) value and hence three different types of biochemical parameters exhibited highly significant effect on different stages of white coloured mushroom P. djamor. Again, amount of biochemical parameters from different stages of white coloured mushroom P. djamor revealed that calculated F (4.268) was found to be slightly higher than tabulated F (3.0069) and hence amount of biochemical parameters from different stages of white coloured mushroom P. djamor was also found significant (Table 2). It can be concluded that the investigated wild edible white coloured mushroom P. djamor is a good source of food in terms of protein and may be cultivated and can be easily consume.

REFERENCES

- Adejumo T. O. and Awosanya O. B. 2005. Proximate and mineral composition of four edible mushroom species from southwestern Nigeria, Afr. J. Biotechnol. 4(10): 1084-1088.
- Alvarez-Parrilla E., de la Rosa L. A., Martinez N. R. and González Aguilar G. A. 2007. Total phenols and antioxidant activity of commercial and wild mushrooms from Chihuahua, Mexico, Ciencia y Tecnol. Aliment. 5(5): 329-334.
- Bahl N 1994. Handbook on Mushrooms, Oxford and IBH Publishing Co., Delhi, India.
- Barros L., Ferreira M. J., Queiros B., Ferreira I.C.F.R and Baptista P 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and antioxidant activities. *Food Chem.* **103**, 413-419.
- Buswell J.A and Chang S.T. 1993. Edible Mushrooms: attributes and applications, *Genetics and Breeding of Edible Mushroom* (eds. Chang S.T., Buswell J.A. and Miles P.G), Grodon & Breach Science Publishers, Amsterdam, pp. 297-306.
- Chang S.T and Hayes W.A 1978. The biology and cultivation of edible mushrooms. Academic Press, London.
- Cheung L.M and Cheung P.C.K 2005. Mushroom extracts with antioxidant ability against lipid peroxidation. *Food Chem.* **89,** 403-409.
- Cheung L.M, Cheung P.C.K and Ooi V.E.C 2003. Antioxidant activity and total phenolics of edible mushroom extracts, Food Chem. 81, 249-255.
- Choi Y., Lee S. M., Chun J., Lee H. B and Lee J 2006. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom, *Food Chem.* **99**, 381-387.
- Cuptapun Y., Hengsawadi D., Mesomya W and Yaieiam S 2010.

 Quality and quantity of protein in certain kinds of edible mushroom in Thailand, *Kasetsart. J. (Nat. Sci.).* 44, 664-670
- Danell E and Eaker D 1992. Amino acid and total protein content of the edible mushroom *Cantharellus cibarius* (Fries), *J.Sci. Food Agric.* **60**, 333-337.
- Ferreira I. C. F. R., Baptista P., Vilas-Boas M and Barros L. 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal:

- individual cap and stipe activity, Food Chem. 100, 1511-1516
- Flegg P.B and Maw G 1977. Mushrooms and their possible contribution to world protein needs, *Mushroom J.* 48, 395-403.
- Georgé S., Brat P., Alter P and Amiot M. J 2005. Rapid determination of polyphenols and vitamin C in plant-derived products, *J. Agric. Food Chem.* **53**, 1370-1373.
- Hafiz F., Begum M., Parveen S., Nessa Z and Azad A.K.M 2003. Study of edible mushroom grown on *Eucalyptus camaldulensis* trunk and under the soil of *Albizzia procera*, *Pak. J. Nutr.* 2(5), 279-282.
- Hassan F.R.H., Medany G.M and Hussein S.D.A. 2010. Cultivation of the king oyster mushroom (*Pleurotus eryngii*) in Egypt, Aus. J. Basic Appl. Sci. 4(1): 99-105.
- Hirano R., Sasamoto W., Matsumoto A., Itakura H., Igarashi O and Kondo K 2001. Antioxidant ability of various flavonoids against DPPH radicals and IDL oxidation, J.Nutr. Sci. Vitaminol. (Tokyo) 47, 357-362.
- Kähkönen M. P., Hopia A. I. Heikki J. V., Jussi-Pekka R., Pihlaja K., Kujala T. S and Heinonene M 1999. Antioxidant activity of plant extracts containing phenolic compounds, *J. Agric. Food Chem.* 47, 3954-3962.
- Kalyoncu F., Erogonul B., Yildiz. H., Kalmiu E and Solak M. H 2010. Chemical composition of four wild edible mushroom species collected from southwest Anatolia, *Gazi Univ. J. Sci.* 23(4), 375-379.
- Khan M.A., Khan L.A., Hossain M.S., Tania M and Uddin M.N 2009. Investigation on the nutritional composition of common edible and medicinal mushrooms cultivated in Bangladesh, Ban. J. Mushrom 3(1), 21-28.
- Kihumbu G.A, Anakalo A.S., Maina S.M. Khare K.B and Sharma H.K 2008. Nutritional composition of *Pleurotus sajor-caju* grown on water hyacinth, wheat straw and corncob substrates, *Res. J. Agric. Biol. Sci.* 4(4), 321-326.
- Lakshmi B., Tilak J. C., Adhikari S., Decasagayam T. P. A and Janardhanan K. K. 2004. Evaluation of antioxidant activity of selected Indian mushrooms, *Pharmaceut. Biol.* 42, 179-185.
- Lang C. A 1958. Simple micro-determination of kjeldahl nitrogen in biological materials, Anal. Chem. 30, 1692-1694.
- León-Guzmán M. F., Silva I. and López M. G. 1997. Proximate chemical composition, free amino acid contents and free fatty acids contents of some wild edible mushrooms from Queretaro, México, *J. Agric. Food Chem.* **45**, 4329-4332.
- Lintzel W 1941. The nutritonal value of edible mushroom protein, Biochem. Acta. 308, 413-419.
- Lo K. M and Cheung P. C. K 2005. Antioxidant activity of extracts from the fruiting bodies of Agrocybe aegerita var. alba, Food Chem. 89, 533-539.
- Lowry O. H., Rosenbrough N. J. Farr A. L and Randoll R. J 1951.

 Estimation of protein with the folin-phenol reagent. *J. Biolog. Chem* 193, 265-275.
- Manjunathan J and Kaviyarasan V 2010. Nutrient distribution from a newly cultivated edible mushroom Lentinus tuberregium (Fr.) Tamil Nadu, India, J. Chem. Pharm. Res. 2(3), 575-578.
- Manzi P., Gambelli L., Marconi S., Vivanti V and Pizzoferrato L 1999. Nutrients in edible mushrooms an inter-species comparative study, Food Chem. 65, 477-482.
- Mata G and Salmons D 2003. Edible mushroom cultivation at the institute of ecology in Mexico, *Micologia Aplicada Internacional* **15**(1), 23-29.
- Mau J. L., Chao G. R. and Wu K. T 2001. Antioxidant properties of methanolic extracts from several ear mushrooms, J. Agric. Food Chem. 49, 5461-5467.

- Mau J. L., Chang C.N., Huang J and Chen C.C 2004. Antixidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia, *Food Chem.* 87, 111-118.
- Omarini A., Henning C., Ringuelet J., Zygadlo J. A and Albertó E 2010. Volatile composition and nutritional quality of the edible mushroom *Polyporus tenuiculus* grown on different agro-industrial waste, *Int. J. Food Sci. Technol.* 45, 1603-1609.
- Oyetayo V.O. and Oyetayo F.L. 2005. Preliminary study of the health promoting potentials of *Lactobacillus fermentum* OVL and *Plerotus sajor-caju* administered to rats, *Pak. J. Nutr.* 4(2), 73-77.
- Petrovska B. B 2001. Protein fraction in edible Macedonian mushrooms, Eur, Food Res. Technol. 212, 469-472.

- Rodrigo R and Bosco C 2006. Oxidative stress and protective effects of polyphenols: comparative studies in human and rodent kidney, *Comprehensive Biochem. Physiol. Part C* 142, 317-327.
- Steel R. G. D. and Torrie T. H. 1980. Principles and Procedures of Statistics, McGraw-Hill Book Company Inc., New York, pp. 481.
- Yang J.H., lin H.C and Mau J.L. 2002. Antioxidant properties of several commercial mushrooms, Food Chem. 77, 229-235.
- Yen G.C and Hung C.Y 2000. Effects of alkaline and heat treatment on antioxidative activity and total phenolics extracts from Hsiantsao (*Mesona procumbens* Hemsl.), Food Res. INt.35, 487-492.

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