



Antioxidant profile of *Agaricus bisporus* and *Calocybe indica*

M. Suganya¹ and M. Suriyavathana^{2*}

¹, Department of Biochemistry, Periyar University, Salem, (TN) - India

², Department of Biochemistry, Sir Sankara Arts and Science College, Kanchipuram, (TN) - India

Abstract

The Button mushroom *Agaricus bisporus*, Milky mushroom *Calocybe indica* are the most common cultivated mushrooms, and they susceptible to a wide range of virus, bacterial and fungal diseases. The modulation of the mushroom used now-a-days in the field of medicine, is the way where this is partly achieved. The aim of this project is to incorporate the Antioxidant profile in dried from of powered samples of the *Agaricus bisporus* and *Calocybe indica*. Among the two mushroom varieties *Agaricus bisporus* show higher level of SOD, GST, while the enzymatic antioxidant content specifically GPX and Catalase is high in *Calocybe indica* mushroom variety. The level of GPx seems to be slightly varied between the *Agaricus bisporus* and *Calocybe indica* mushroom types. Both *Agaricus bisporus* and *Calocybe indica* shows good store of Enzymatic Antioxidant. By comparing the vitamin levels both *Agaricus bisporus* and *calocybe indica* mushroom varieties exhibited higher content of ascorbic acid. The level of α -tocopherol is found to be higher in *Calocybe indica* mushroom than *Agaricus bisporus*.

Key-Words: *Agaricus bisporus*, *Calocybe indica*, Antioxidant, Enzymatic & Non- Enzymatic

Introduction

Mushroom a group known as fungi lacks chlorophyll and cannot therefore make its own food. Among the fungi, mushrooms have been used for untold centuries as food and medicine. Edible and medicinal mushrooms not only convert the huge lignocellulosic biomass waste in human food, but most remarkably can produce notable mycopharmaceuticals, myconutriceuticals and myocosmeceuticals for many years mankind has benefited source of drugs and herbal remedies, the major source of pharmaceuticals and medicinal foods.(6)

A mushroom is the fleshly, spore bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. Mushroom describes a variety of gilled fungi, with or without stems and the term is used even more generally to describe both the fleshy fruiting bodies of some Ascomycota and the or leathery fruiting bodies of some Basidiomycota, depending upon the context form deviating from the standard mushroom morphology usually have more specific names such as "puffball" "stinkhorn" and "morel" and gilled mushroom are often called "*agaricus*". Edible mushroom are consumed by humans for their nutritional are occasionally medicinal value as comestibles. Mushroom consumed for health reason are know as medicinal mushroom. (7)

A typical mushroom is the lobster mushroom, which is a deformed, cooked-lobster coloured parasitized fruit body of a *Russula* or *Lactarius* coloured and deformed by the myoparasitic ascomycete *hypomyces lactifluorum*. The term mushroom is more one of common application to macroscopic fungal fruiting bodies than one having precise taxonomic meaning. There are approximately 14,000 described species of mushroom. The button mushroom *Agaricus bisporus* Imbach is the most common cultivated mushroom and is consumed throughout the world. Commercial shows produce substantial yield and exhibit attractive morphology and texture to a variety of viral, bacterial and fungal diseases.(6)

Agaricus bisporus

Agaricus bisporus known widely as the common mushroom, button mushroom, white mushroom, table mushroom, cremini, crimini mushroom, swiss brown mushroom, roman brown mushrooms, and Italian brown or cultivated mushroom is an edible mushroom. *Agaricus bisporus* is cultivated is more than 70 countries and is one of the most commonly and widely consumed mushroom.(6)

Calocybe indica

Calocybe indica is a small genus of about 40 species of mushroom including St.George's mushroom which is edible and milky mushroom and is cultivated in India. *Calocybe indica* commonly known as the milky mushroom is a relatively new introduction to the world

* Corresponding Author

E.mail:suriyaveda@yahoo.co.in, sugu65@gmail.com
Mob. +91-9039924964

of edible Cellulose is the most widely distributed carbohydrate of the plants. Edible and medicinal mushrooms not only convert the huge lignocellulosic biomass waste into human food, but most remarkably can produce notable mycopharmaceuticals, myconutriceuticals and mycosmeceuticals for many years has benefited from green plants as a source of drugs and herbal remedies.(6)

Material and Methods

The fungal species *Agaricus bisporus* and *Calocybe indica* was collected and washed well in tap water first and then with the distilled water. The cleaned tubers were sliced and allowed for the complete shade drying and then made to fine powder with homogenizer. The individual sample preparation for each analysis were explained.

The mushroom samples of *Agaricus bisporus* and *Calocybe indica* was crushed and made to fine powder using homogenizer. About 1.0 g of clear dried sample was taken along with 10ml ethanol and mashed well in a homogenizer and filtered, then used for analysis.

Enzymatic antioxidants assay of superoxide dismutase

0.5 ml of homogenate was diluted to 1 ml with water. Then 2.5 ml of ethanol and 1.5 ml chloroform (all reagents chilled) was added. This mixture was shaken for 1 minute at 40C and then centrifuged. The enzyme activity in the supernatant was determined.

Assay of catalase

To 0.9 ml of phosphate buffer, 0.1 ml of homogenate and 0.4 ml of hydrogen peroxide were added. After 60 sec. 2.0 ml of dichromate acetic acid mixture was added. The tubes were kept in boiling water bath for 10 minutes and the colour developed was read at 620 nm.

Assay of glutathione peroxidase

To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of homogenate were added. To the mixture, 0.2 ml of glutathione followed by 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37oC for 10 minutes along with a tube containing all the reagents except sample. After 10 minutes the reaction was arrested by the addition of 0.5 ml of 10% TCA, centrifuged and the supernatant was assayed.

Assay of glutathione-s- transferase

The reaction mixture contained 1.0 ml of phosphate buffer, 0.1 ml of CDNB, 0.1 ml of homogenate and 0.7 ml of distilled water. The reaction mixture was incubated at 37oC for 5 minute then the reaction was started by the addition of 0.1 ml of 30mM glutathione. The absorbance change was read at 340nm for 5

minutes. Reaction mixture without the enzyme was used as the blank.

Activity of glucose-6-phosphate dehydrogenase

The incubation mixture contained 1 ml of buffer, 0.1 ml of magnesium chloride, 0.1 ml of NADP+, 0.5 ml of phenazine methosulphate, 0.4 ml of the dye solution and the requisite amount of the enzyme extract. The mixture was allowed to stand at room temperature for 10 min to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of glucose-6-phosphate. The absorbance was read at 640nm against water blank at one min intervals for 3-5 min in a UV spectrophotometer. The activity of the enzyme was calculated in units by multiplying the change in OD/min by the factor 6/17.6, which is the molar extinction co-efficient of the reduced co-enzyme.

Non – enzymatic anti oxidants estimation of reduced glutathione

A known weight of tissue was homogenized in phosphate buffer. From this 0.5 ml was pipetted out and precipitated with 2.0 ml of 5% TCA. 1.0 ml of the supernatant was taken after centrifugation and added to it 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer. The yellow colour developed was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer.

Estimation of ascorbic acid (Vitamin c)

0.5 ml of tissue homogenate was mixed thoroughly with 1.5 ml of 6% TCA and centrifuged for 20 minutes at 3500 g. To 0.5 ml of the supernatant, 0.5 ml of DNPH reagent was added and mixed well. The tubes were allowed to stand at room temperature for an additional 3 hours. Removed, placed in ice-cold water and added 2.5 ml of 85% sulphuric acid and allowed to stand for 30 minutes. A set of standards containing 10-50g of ascorbic acid were taken and processed similarly along with a blank, containing 0.5 ml 4% TCA. The colour developed was read at 530nm

Results and Discussion

Among the two mushroom varieties *Agaricus bisporus* show higher level of SOD, GST, while the enzymatic antioxidant content specifically GPX and Catalase is high in *Calocybe indica* mushroom variety. The level of GP₆D seems to be slightly varied between the *Agaricus bisporus* and *Calocybe indica* mushroom types. Both *Agaricus bisporus* and *Calocybe indica* shows good store of Enzymatic Antioxidant.

By comparing the vitamin level among the *Agaricus bisporus* and *Calocybe indica* varieties. Both *Agaricus bisporus* and *calocybe indica* mushroom variety exhibited high content of ascorbic acid kingdom. The level of Vitamin C varied between the varieties is very narrow. The level of α -tocopherol is found to be higher

Calocybe indica mushroom. The level of GSH higher in *Agaricus bisporus* than *Calocybe indica* mushroom. From the present study it could be that concluded both the *Agaricus bisporus* and *Calocybe indica* varieties possess Enzymatic and Non Enzymatic activity through *Agaricus bisporus* and *Calocybe indica*.

Acknowledgement

We are grateful to Department of Biochemistry, Periyar university, Salem-11, Tamil Nadu, For supporting the study.

References

1. Baker H. Frankel, O. De Angelis B. and Feingold S. (1980). Plasma α -tocopherol man at various time intervals after ingesting free or acetylated tocopherol. *Nutr. Rep. Int.*, 21: 531 - 536.
2. Ellis H.A. and Kirkman H.N. (1961). *Proc. Soc. Exp. Biol. (N.Y.)*: 106 - 607
3. Ellman G.L. and Boyne A.F. (1972). A methodology for analysis of tissues sulfhydryl components. *Anal. Biochem.* 46(2): 639 - 53.
4. Habig W.H., Pabst M.J. and Jakoby W.B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 246: 7130 - 7139.
5. Kakkar P. and Vishwanathan P.N. (1984). A modified Spectrophotometric assay of Superoxide dismutase. *Indian Journal of Biochem. Biophys.* 21: 130 - 132.
6. Law, S.k. and T.B.N.g, 2001. Anti fungal and antifungal activities from jaiting bodies of edible mushrooms. *Biochem. Biophys. Res. Commu.* 285: 1071-1075.
7. National Research Centre for mushroom, chabaghat, 173 213.
8. Omaye S.T., Turnbull T.D. and Sauberlich H.C. (1979). Selected method for the determination of ascorbic acid in animal cell tissues and fluids. *Methods Enzymol* 62: 3 - 11.
9. Rotruck J.J., Pope A.L., Gantter H.E. and Swanson A.B. (1973). Selenium: Biochemical role as a component of glutathione Peroxidase. *Science*, 179: 588 - 590.
10. Sinha A.K. (1972). Colorimetric assay of catalase. *Anal. Biochem.* 47: 389 - 394.
11. Vural H., Aksoy N. and Ozbilge H. (2004). Alterations of oxidative status in human cutaneous leishmaniasis. *Cell Biochem. Funct.* 22: 153-156.

Table 1: Level of enzymatic antioxidants

Species	Glutathione peroxidase ($\mu\text{g}/\text{mg}$)	Glucose-6-Phosphate D'se ($\mu\text{g}/\text{mg}$)	Catalase ($\mu\text{g}/\text{mg}$)	GST ($\mu\text{g}/\text{mg}$)	SOD ($\mu\text{g}/\text{mg}$)
Button mushroom	1545.4 \pm 0.22	0.2185 \pm 0.04	247.9 \pm 0.27	2161.9 \pm 0.191	908.1 \pm 0.238
Milky mushroom	1800 \pm 2.38	0.2104 \pm 0.00018	330.5 \pm 0.28	1900.5 \pm 0.170	878.57 \pm 0.029

Table 2: Level of non-enzymatic antioxidants

Estimation	Vit-E ($\mu\text{g}/\text{g}$)	Vit-C ($\mu\text{g}/\text{g}$)	Reduced glutathione ($\mu\text{g}/\text{g}$)
Button mushroom	44.4 \pm 1.22	86 \pm 0.78	12.92 \pm 0.42
Milky mushroom	42.2 \pm 0.122	84 \pm 0.82	10.77 \pm 0.11

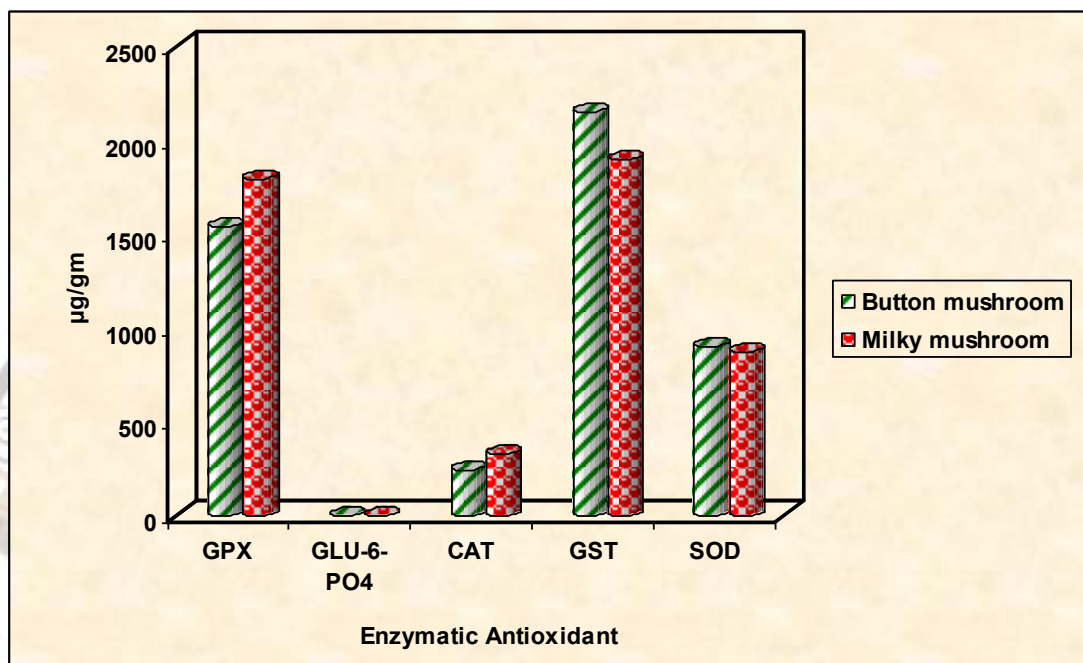


Fig. 1: Level of enzymatic antioxidants

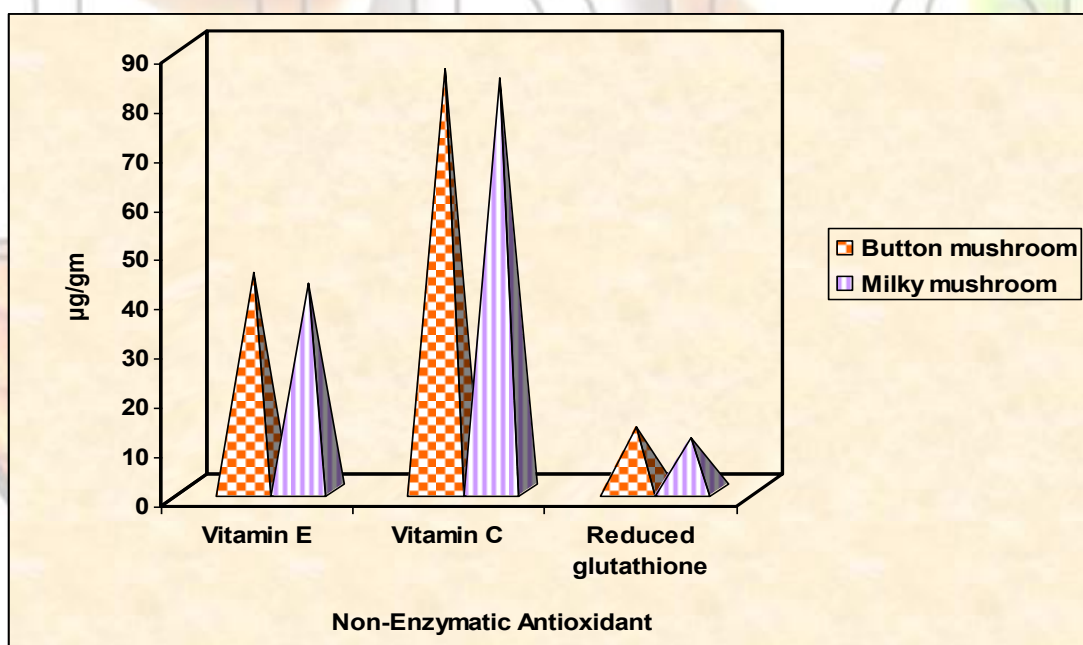


Fig. 2: Level of non-enzymatic antioxidants