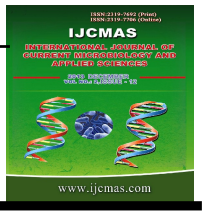
Int.J.Curr.Microbiol.App.Sci (2013) 2(12): 255-263

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 12 (2013) pp. 255-263**
http://www.ijcmas.com



Original Research Article

Purification and characterization of Catalase enzyme from *Agaricus bisporus*

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K e y w o r d s

Agaricus bisporus; Sephadox-75; Polyacrylamide gel electrophoresis.

A B S T R A C T

Agaricus bisporus widely known as the common edible mushroom, it is cultivated more than 70 countries and is one of the most commonly consumed mushroom. The occurrence of high amounts of proteins, carbohydrates, fibers, and low fat contents are often referred in the literature in relation to their nutritional value. Catalase is a ubiquitous enzyme found in aerobic organisms. The aim of this project is to determine the catalase activity in the *Agaricus bisporus.* Catalase has been purified to homogeneity in the analytical ultracentrifuge, DEAE cellulose, Sephadox-75. Polyacrylamide gel electrophoresis revealed molecular weight 45 kDa for Catalase, which constituted 90% of the total protein of the stained gel, suggesting that the native enzyme is tetrameric.

Introduction

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 leathery fruiting bodes 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"*.

The common white mushroom, *Agaricus bisporus*, is a fungus of the order Agaricales. This order contains over 13,000 fungi, which are readily recognizable as "gilled mushrooms." The above□ground spore□body of agaric fungi all share common features: they are fleshy, with a *stipe* (stem), a *pileus* (cap), and *lamellae* (gills). They often have an *annulus* (ring) which is a remnant of the *veil* that protects the gills during maturation. It is widely known as the common mushroom, button mushroom and white mushroom. It is cultivated in more than 70 countries and is one of the most commonly consumed mushroom.

Edible mushroom is consumed by humans for their nutritional**.** Mushrooms have been used as food and food-flavoring material in soups and sauces for centuries, due to their unique and subtle flavor. The occurrence of high amounts of proteins, carbohydrates, fibers and low fat contents are often referred in the literature in relation to their nutritional value. Regarding their medicinal value, mushrooms are effective as antitumor, antibacterial, antiviral and hematological agents and in immune modulating treatments (Wasser & Weis, 1999); Yang, Lin, & Mau, 2002). Mushroom species have been shown to possess antioxidant capacity in in-vitro systems (Ribeiro et al., 2006). Like other matrices containing antioxidant compounds, e.g. phenolics (Bendini et al., 2006; Quezada et al., 2004), organic acids (Mato et al., 2003) and alkaloids. Mushrooms can be used both as a food supplement and in the pharmaceutical industry.

Catalase is a ubiquitous enzyme found in aerobic organisms. It efficiently catalyzes the decomposition of hydrogen peroxide to oxygen and water together with other enzyme systems, protects cells against the harmful effects of reactive oxygen species such as superoxide anions, hydrogen peroxide, and hydroxyl radicals. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Enzymes have been significant industrial products for more than a hundred years. For example, a therapeutic enzyme was described as a part of replacement therapies for genetic deficiencies. However, the range of potential application is increasing rapidly. In this investigation it has also been planned to impregnate antioxidant enzyme purification from edible mushroom and its physio-chemical properties for effective therapeutic treatment.

Materials and Methods

Collection of sample

Agarics bisporus were collected from the Tamilnadu Agricultural university, Coimbatore and washed well in tap water first and then with the distilled water. The cleaned tubers were sliced and kept in refrigerator.

Catalase enzyme extraction

The sliced mushroom tubers were homogenized by pre-chilled motor and pestle. Enzyme extraction was prepared with 1000ml of cold 0.067M phosphate buffer (pH 7) for 200g of mushroom tubers. The homogenate was centrifuged at 10000 rpm for 30min and supernatant was collected. The sediments were mixed with cold phosphate buffer, allowed to stand in cold condition with occasional shaking. Then the sediment containing buffer was subjected to centrifugation once again to collect supernatant. The supernatant was used as a source of enzyme.

Protein estimation (Lowry et al 1951 method)

Protein content of enzyme extract is usually determined by this method, using crystalline Bovine Serum Albumin as standard. The blue colour was developed by the reduction of the phosphomolybdic phosphotungstic components in the Folin-ciocalteau reagent and biuret reaction of the protein with the alkaline cupric

tartarate were measured at 660nm by spectrophotometer. 2ml and 0.4ml of mushroom supernatants were used to determine the protein content.

Catalase assay
(Sadasivam and Manickam, 2008)

The supernatant of *Agaricus bisporous* was used to determine the catalase activity by monitoring the decrease in A_{240} resulting from the elimination of H_2O_2, using a Hitachi U-3210 spectrophotometer. 3ml of Hydrogen peroxide –Phosphate buffer is taken in a cuvette and mixed in 0.01-0.04ml of sample. Read against a control containing enzyme solution but containing H_2O_2 free PO_4 at the wavelength of 240nm in spectrophotometer. The time required from 0.45 to 0.40 is noted. Thus the decrease value is used for calculation.

Ammonium sulphate precipitation and dialysis

Ammonium sulphate precipitation of antioxidant enzyme was done in an ice bath using the finely grounded ammonium sulfate. The powder was weighed and added slowly to the extract by constant stirring to ensure completely solubility, and the solution was centrifuged at 10000rpm for 30min at 4^0C. Different precipitation steps were carried out for Calatase enzyme precipitation (45-90%) and precipitates were collected. The precipitate was dialyzed against 50mM potassium phosphate (pH 7) for 24hr by changing the buffer thrice. The dialyzed fraction was used for further analysis.

Purification of catalase enzyme
(Tony ching *et al*., 1973)

DEAE-cellulose column (1 by 3) equilibrated with 50mM pottasium phosphate buffer (Buffer A- pH 7). 30µl of the dialyzed enzyme solution was applied to the column and washed with 4 ml of Buffer A. Elution also done by distilled water. Active fraction detected for catalase activity by monitoring the decrease in $A_{240.}$ The fractions were collected and pooled for subsequent purification in sephadex G-75 column (0.5 X 5 cm) that had been equilibrated with Buffer A (pH 7). The active fractions (8 ml) were diluted to 32 ml with the Buffer A in order to reduce the salt concentration. The fraction was then applied to the column and washed with the 3 ml of Buffer A (pH 7). Elution was performed with distilled water (total volume 15 ml). The volume of 1 fraction was 1 ml. Then the enzyme was stored at 4°C.

Molecular weight determination of purified catalase

The standards used to make a plot of log molecular weight *versus* mobility of the protein band were: lysozyme (14kDa), pepsin (36kDa), egg albumin (45kDa), bovine serum albumin (67 kDa), phosphorylase B (94kDa), ovalbumin (43kDa), trypsin inhibitor (20100 kDa) and_β-lactalbumin (14 400 kDa).

Sodium dodecylsulfate-polyacrylamide gel (SDS-PAGE) electrophoresis of purified catalase

SDS PAGE is performed using a 10 % separating gel, 4 % stacking gel without-mercaptoethanol. The samples were heated for 5 min at 100 ºC in capped vials with 1% (w/v) SDS in the presence of β-mercaptoethanol. Electrophoresis was performed at a constant 125V (8.0 mA) for 4 h in Tris-HCl buffer pH 8.3. After electrophoresis, proteins in the separating gel were made visible by staining with Coomassie Brilliant Blue R-250.

Analysis of pH profile

The activity of catalase for different pH was obtained using 0.067M potassium phosphate buffer (pH 3 to pH 8). In each pH buffer (1 ml), 30µl of enzyme solution was mixed and kept for overnight at 4 °C. After this period, enzyme activity of samples was analyzed under the standard assay condition.

Analysis of thermostability

10µl of catalase was placed in water bath at a temperature of 10, 20, 30, 40, 50 and 60°C for periods of 60 min. Enzyme activity after treatment was analyzed under the standard assay condition.

Storage stability

30µl of catalase was mixed with 1ml of potassium phosphate (pH7) buffer and stored at the 4 °C. Enzyme activity was analyzed every day up to 1 week in order to find out the storage stability of enzymes.

Result and Discussion

Protein estimation

Standard graph was plotted for bovine serum albumin using spectrophotometric reading at 660 nm. Total protein obtained for *Agaricus bisporus* were 2.05 mg/ml for 20 µl and 4.3 mg/ml for 40 µl sample (Table 1). Ouzouni *et al.*, (2009) reported the fruiting bodies of mushrooms contain about 56.8% carbohydrate, 25.0% protein, 5.7% fat and 12.5% ash on a dry weight basis. Previous research on the use of mushrooms in human nutrition, especially on their nutritive and curative aspects, proved without doubt that mushrooms are extremely important (Grlic, 1980). Similarly, protein content of edible mushrooms taken for analysis revealed that the presence of total protein about 2.05 µg/ml in *Agaricus bisporus.*

Catalase assay

Catalase assay was performed for the supernatant of mushroom extract, enzyme activity were measured at 240nm wavelength. Time taken for the reduction of A_{240} values from 0.45-0.4 was noticed. The enzyme activity for *Agaricus bisporus* was calculated as 12.05 U/ml. Previous studies had shown catalase exist in multiple forms in several plants such as tobacco, saffron, cotton, mustard, maize, wheat, sunflower, castor bean, spinach, pepper, loblolly pine and kohlrabi (Havir and McHale, 1987). In the present study, catalase enzyme from *Agaricus bisporus* was 12.05 U/ml and specific activity of 14.3 U/mg. Catalase activity of *Agaricus bisporus* was determined in the presence of hydrogen peroxide.

Purification and molecular weight of catalase from *Agaricus bisporus*

The enzyme eluted as a single species in the initial DEAE-cellulose chromatography step. Additional purification of the enzyme was achieved by gel filtration on Sephadex G-75. Table 2 summarizes the results of each step of the catalse purification. The enzyme was purified about 51.07-fold, with a final specific activity of 14.3U/mg. The overall recovery of the purification was 14%. The molecular mass of catalase in dialysate revealed about 43kDa and purified catalase 45 kDa (Fig.1).). The peak specific activity of catalase was 14.3 U/mg was observed for *Agaricus bisporus*. There was rise in specific activity in each

purification step. Loewen and Switala (1987) were observed the rise of specific enzyme activity in every purification step. The native PAGE method developed by Hedrick and Smith (1968) provides estimation for proteins analyzed on gels of successive high acrylamide concentration. As it has been mentioned above, the molecular weight of catalase T is between 225 - 250 kD, and for catalase A – considerably lower 170 - 190 kD. The molecular weight of purified catalase of *Agaricus bisporus* was 45 kDa. Since the separated enzyme appeared as a single band, it was concluded catalase enzyme to be tetrameric. From the reported data, it can be concluded that catalase varies in its MW depending upon the source from which it is isolated.

Effect of pH on catalase activity

The pH profile on the activity of catalase enzyme from *Agaricus bisporus* mushroom was shown in fig. 2. The optimum pH for the strains of *Agaricus bisporus* was 7.5 and had a minimum activity at pH 3.5.

Effect of temperature on catalase activity

Purified catalase from *Agaricus bisporus* was stable in potassium phosphate buffer (pH 7) for more than 4 days at 4°C, as a decrease in activity observed after 5 days (Fig. 3). After being heated at 10-30°C for 60 minutes, catalase from *Agaricus bisporus* retained 26.98 units/ml of the

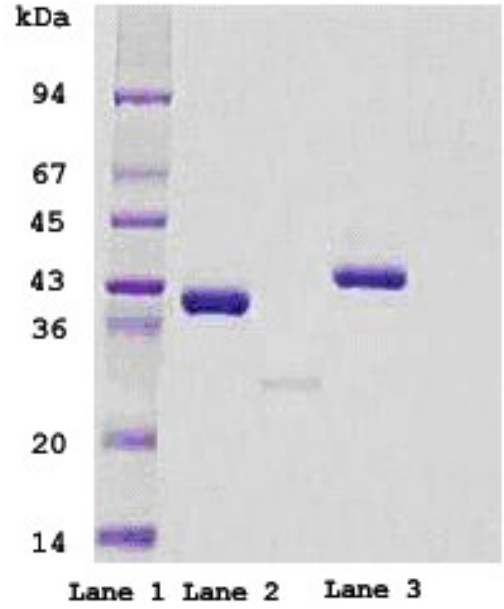
Table.1 Total protein content of *Agaricus bisporous*

Sample volume (µl)	***Agaricus bisporus* (µg/ml)**
20	2.05
40	4.3

Table.2 Purification of catalse from *Agaricus bisporus*

S.No	**Purification step**	**Total amount of protein (mg)**	**Total activity (U)**	**Specific activity (U/mg)**	**Recovery (%)**	**Purification (fold)**
1	Supernatant	430	121	0.28	100	1
2	Dialysate	92	82	0.89	67.7	3.1
3	DEAE- cellulose	87	52	1.67	42.9	5.9
4	Sephadox G-75 fraction	1.2	17.2	14.3	14	51.07

Fig.1 Molecular mass of purified catalases



Lane 1 Marker
Lane 2 *Agaricus bisporous* catalase (dialysat
Lane 3 *Agaricus bisporous* catalase

Figure.2 pH stability of catalase from *Agaricus bisporus*

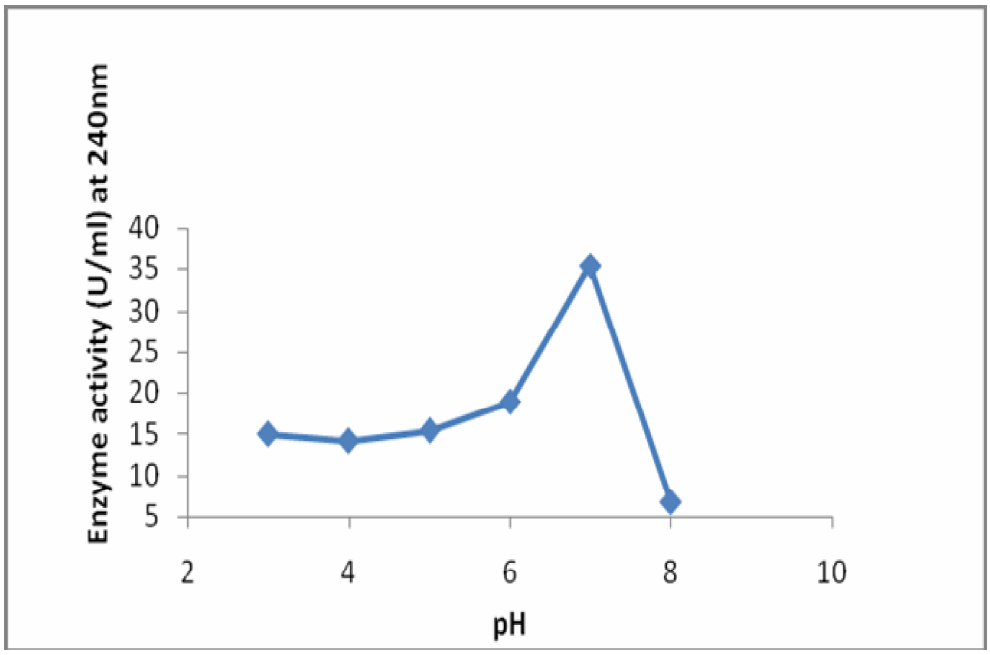


Figure.3 Storage stability of catalase from *Agaricus bisporus*

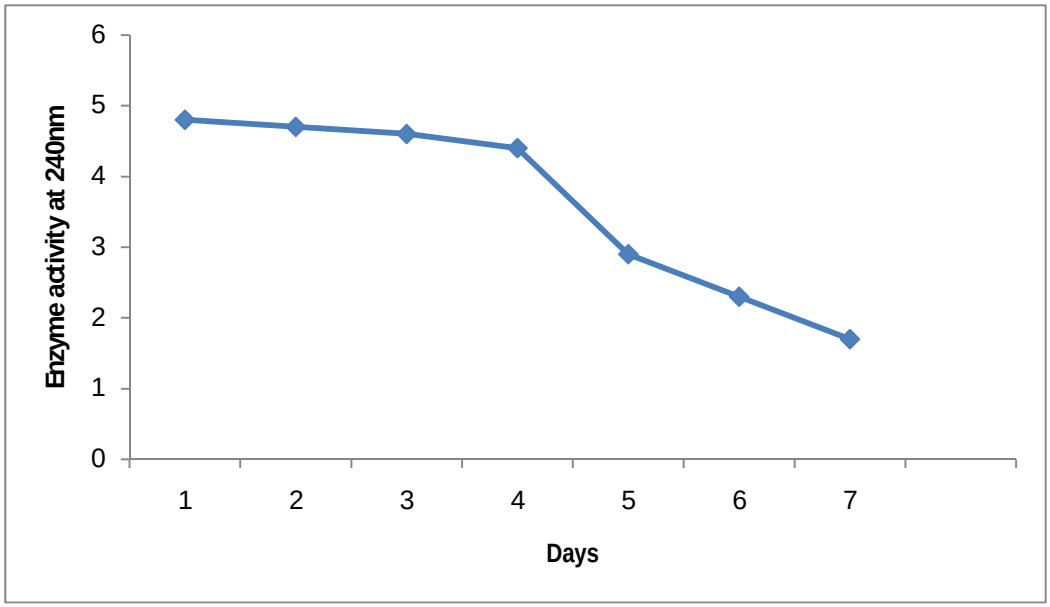
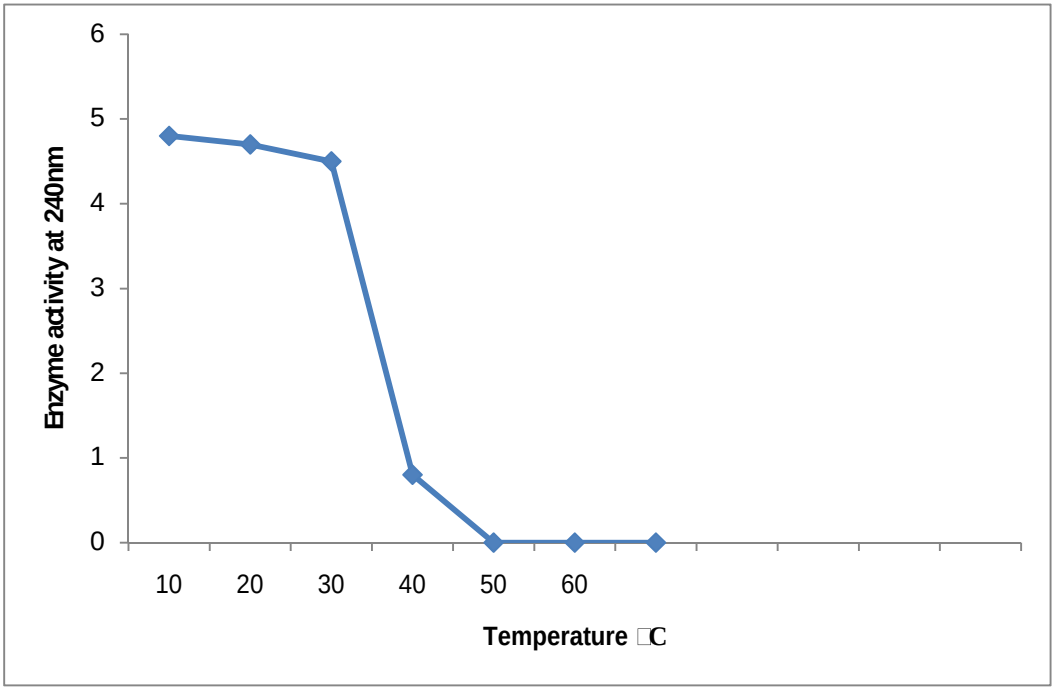


Figure.4 Temperature stability of catalase from *Agaricus bisporus*



enzyme activity measured at 30°C for 60 minutes but became completely inactive after treatment between 40-60 °C for 60 minutes. The thermostability of *Agaricus bisporus* catalase was shown in fig.4 .

Noted that the most interesting characteristics of catalase from mushroom was the optimum pH and temperature. The optimum temperature and optimum pH for purified catalase from *Agaricus bisporus* on enzymatic reaction were 30°C and pH 7 respectively. Similar temperature and pH has also been reported by Aydemir and Kuru (2003) on catalase enzyme of blood erythrocytes.

In this investigation antioxidant enzyme purification from edible mushroom may be used for effective therapeutic treatment. Many medicinal properties have been attributed to mushroom catalases including inhibition of platelet aggregation reduction of blood cholesterol concentrations prevention or alleviation of heart disease and reduction of blood glucose levels, also prevention or alleviation of infections caused by bacterial, viral, fungal and parasitic pathogens. Consumption of mushroom containing phytochemical with potential antioxidant properties can reduce the risk of human disease.

Acknowledgement

We acknowledge our profound gratitude to the Department of Microbiology, Nehru Arts and Science College, T.M.Palayam, Coimbatore for providing the facilities for research work. We are highly indebted to Dr. Anirudhan (Prinicipal) Dr. J. Rathinamala, Dr.T.Balasaravanan Mr.V.Shanmugham and Dr.Meenatchisundaram [Associate Professors] Nehru Arts and Science College, T.M.Palayam, Coimbatore for their valuable help to complete this work.

References

Aydemir and Kuru. 2003. "Purification and characterization of Catalase Chicken Erythrocytes and the effect of Various Inhibitors on Enzyme Activity" . Turk. J. Chem. **27:** 85- 97.

Bendini, A., Cerretani, L., Pizzolante, L., Toschi, T. G., Guzzo, F., Ceoldo, S., et al. 2006. Phenol content related to antioxidant and antimicrobial activities of Passiflora spp. extracts. European Food Res. Technol. 223: 102–109.

Grlic, LJ. 1980. Wild growing edible plants. Education, Zagreb (*Samoniklojestivo bilje*. Prosvjeta, Zagreb) (In Croatian).

Havir, E.A. and McHale, N.A. 1990. Purification and characterization of an isozyme of catalase with enhanced-peroxidatic activity from leaves of *Nicotiana sylvestris*. Arch Biochem Biophys. 283: 491-495.

Hedrick and Smith. 1968. "Size and charge separation and estimation of molecular weights of proteins by disc gel electrophoresis." Archi. Biochem.Biophy.126: 155-164.

Loewen, J. Switala, M. Smolenski, B.L. and Triggs-Raine. 1987. .Biochem Cell Biol. 68:1037–1044.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with Folin phenol reagent . J.Biol.chem.193 : 265.

Mato, I., Huidobro, J., Simal-Lozano, J., and Sancho, M. T. 2003. Significance of nonaromatic organic acids in honey. J. Food Protect. 66: 2371–2376.

Ouzouni, P. K., Petridis, D., Koller, W.D. and Riganakos, K. A. 2009. Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. Food Chem. 115: 1575-1580.

Quezada, N., Ascensio,M., Del Valle, J.

M., Aguilera, J. M., & Go´mez, B. 2004. Antioxidant activity of crude extract, alkaloid fraction and flavonoids fraction from boldo (Peumus boldus Molina) leaves. J. Food Sci. 69: 371–376.

Ribeiro, B., Rangel, J., Valenta˜o, P., Baptista, P., Seabra, R. M. and Andrade, P. B. 2006. Contents of carboxylic acids and two phenolics and ntioxidant activity of dried portuguese wild edible mushrooms. J. Agricult. Food Chem. 54: 8530–8537.

Sadasivam, S., Manikam, A. 2008. Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd. New Delhi.3*:* 99-100

Tony ching, M.S. and Gordin, K . 1973. "Purification and properties of the Catalase of Bakers's yeast." The J. Biol.Chem. 248: 2880-2893

Wasser, S. P. and Weis, A. L. 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspective (review). Inter. J. Med. Mushrooms. 1: 31–62.

Yang, J.-H., Lin, H.-C., and Mau, J.-L. 2002. Antioxidant properties of several commercial mushrooms. Food Chem. 77: 229–235.