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SHORT COMMUNICATION

Enzymatic Antioxidative Properties of Black Oak Mushroom Mycelial Mat (*Lentinus edodes* (Berk.) Peg.)

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This study was undertaken to investigate the organic and inorganic additives of antioxidant activity in the shiitake mushroom (*Lentinus edodes*). In this experiment, enzymatic antioxidants that are CAT, SOD, GPX, POX, GST and GTR were study. The medium amended with calcium carbonate recorded significantly high level of enzymatic antioxidants, compared to non-amended medium. This was followed by the additives sawdust + corn flour < sawdust + rice bran < sawdust. This work suggested that calcium carbonate could be used as a suitable additive to enhance the production of antioxidative substances in the mushroom food industry.

Key words: *Lentinus edodes*, antioxidant, Calcium carbonate, sawdust, Corn flour, rice bran

Medicinal mushrooms have an established history of use in traditional oriental therapies. Mushrooms have been used for many years in oriental culture as tea and nutritional food because of their special fragrance and texture (Manzi et al., 1999). The black oak mushroom is best known with its Japanese name- Shiitake, Chinese name- Xiangu, and French name- Lintin. It is the second most important edible mushroom in the world. This mushroom has been cultivated in Japan, Thailand, Korea, Brazil, and China for about 200 years (Royse, 2005). Among the countries, Japan is the major producer of shiitake mushroom in the world with annual production of 7.5 million tonnes (Smith et al., 2002). The great interest in shiitake's commercialisation is due to its unique flavour, taste, nutritive value, and medicinal properties. The antioxidants present in biological and food materials have attracted considerable interest because of the presumed safety, nutritional, and therapeutic effects (Ames et al., 1993). They also play an essential role in the prevention of cardiovascular diseases (Fuhrman et al., 1993), cancers (Renaud et al., 1999) and neuro-degenerative diseases, the most well known of which are Parkinson's and Ahlzeimer's diseases (Okuda et al., 1992; Clarke, 1999; Joseph et al., 1999), inflammation (Joseph et al., 1999) and cutaneous ageing (Prior and Coa, 2000).
Shiitake mushroom contains several therapeutic actions like antioxidant property, carried by the diversity of its compounds (Anil, 2006; Yen et al., 2007). The objective of this study was to determine the antioxidant properties of *L. edodes*.

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Isolation: Shiitake mushroom fungus, viz., *L. edodes* was isolated on potato-dextrose agar (PDA) medium from freshly harvested sporophores. The sporophores of individual mushroom fungi were first swabbed with 80 per cent ethyl alcohol to remove external microbial contaminants. At the junction of pielus and stripe, tissue bits taken separately, using a sterile foreceps, were surface disinfected with hypochlorite (commercial) for 5 min and rinsed with three changes of sterile distilled water. The surface sterilized tissue bits were placed on PDA in Petri dishes and incubated at laboratory temperature (25 ± 3ºC) for 10 days.

Sample preparation: Potato-dextrose broth (50 ml) was dispensed with additives in 100 ml Erlenmeyer flask and sterilized at 15 lbs pressure for 1h. After cooling, the flask was inoculated with a disc (9 mm dia) taken from the peripheral growth of ten days old PDA culture of the mushroom fungus. They were incubated at laboratory conditions (25 ± 3ºC) for complete coverage of mycelia. After extracting, Whatman No.1 filter paper was dried at 80ºC for 6 h. The dried Whatman No.1 filter paper was used for the analysis of enzymatic and non enzymatic antioxidant activity. Each sample (dried) was weighed (5 g) and transferred into a beaker. Methanol was added to the samples in the ratio 1:10 and stirred for 1 h. The extract was left overnight. The extraction was separated from the residue by filtration through Whatman No.1 filter paper. The residue was re-extracted twice and two extracts were combined. The sample was lyophilized and the residual solvent extract was removed under reduced pressure at 40ºC using a rotary evaporator. Extracts were

Table 1. Effect of additives on enzymatic antioxidative substances in *L. edodes*

Additives (2 %)	CAT	SOD	GPX	POX	GST	GTR
Organic						
Sawdust	8.16 [d]	6.38 [c]	23.47 [d]	12.01 [d]	36.99 [c]	15.78 [d]
Rice bran	7.88 [h]	6.17 [f]	23.18 [h]	11.26 [g]	36.26 [g]	15.47 [h]
Corn flour	7.90 [g]	6.12 [g]	23.31 [f]	11.07 [h]	36.16 [h]	15.58 [g]
Sawdust + rice bran	8.22 [c]	6.28 [d]	23.58 [c]	12.10 [c]	37.03 [c]	16.04 [b]
Sawdust + corn flour	8.26 [b]	6.47 [b]	23.65 [b]	13.00 [b]	37.74 [b]	15.94 [c]
Inorganic						
Calcium carbonate	8.69 [a]	6.95 [a]	24.12 [a]	13.31 [a]	38.13 [a]	16.20 [a]
Gypsum	8.07 [e]	6.24 [e]	23.35 [e]	11.68 [e]	36.57 [e]	15.66 [f]
Lime	7.99 [f]	6.19 [f]	23.23 [g]	11.40 [f]	36.48 [f]	15.70 [e]
Calcium carbonate + gypsum	8.59 [a]	6.83 [a]	24.01 [a]	13.26 [a]	38.05 [a]	16.12 [a]
Control	7.17 [i]	6.01 [h]	23.03 [i]	11.69 [e]	36.78 [d]	13.10 [i]

Values are expressed as Mean of three replicates. Means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test (DMRT).

Units: CAT -1 µ mole of H2O2 decomposed / min / mg protein, SOD - 1 µ mole of inhibition of 50 per cent nitrite formation / min / mg protein ,

GPX-1 µ mole of glutathione utilized / min / mg protein, POX- 1 µ mole of pyrogallol oxidized / min / mg protein, GST - 1 µ mole of CDNB conjugate formed / min / mg protein,

GTR - 1 µ mole of glutathione utilized / min / mg protein.

produced in triplicates and used for the assay of antioxidant activity (Anil, 2006).

Estimation of enzymatic antioxidant: The mean CAT activity (µmol of H_2O_2 consumed/min/mg protein) was assayed following the method described by Sinha (1972). The mean activity of SOD (units/ min/ mg protein) was determined by the method of Deepika Kumari and Varenyam Achal (2008), in which one unit was considered to be the amount of enzyme that inhibited pyrogallol autooxidation by 50 per cent. Mean GPX activity (µmol of glutathione oxidized/ min/ mg protein) was evaluated by following the method described by Rotruck et al. (1973). Mean POX activity (µmol of pyrogallol oxidized/ min/ mg protein) of the mushroom extracts was measured according to the method described by Sadasivam and Manickam (1992). The mean activity of GST (µmol of CDNB conjugate formed/ min/ mg protein) methanolic extracts was estimated by a spectrophotometric assay, based on procedures described by Lin (1999). The mean GTR (µmol of glutathione utilized / min/ mg protein) assay was determined according to the method described by Lin (1999). All experiments were done in three replicates except first experiment (Ten replicates), and mean values are presented. Statistical analysis was performed on the data by Dunccan's Multiple Range Test (DMRT) with means followed by a common letter are not significantly different at the 5% level by DMRT.

The results of the present experiment clearly indicate that among the additives, calcium carbonate was significantly increased the enzymatic antioxidative substances, compared to other additives. The additive calcium carbonate recorded maximum amount of enzymatic antioxidants *viz.,* CAT, SOD, GPX, POX, GST, and GTR; 8.69 µmol, 6.95 µmol, 24.12 µmol, 13.31 µmol, 38.13 µmol and 16.20 µmol, respectively. This was followed by (Table 1). A number of plant constituents have been recognised to have positive effects when tested against the oxygen reactive compounds in biological systems. Many plant by-products, such as peanut hulls, carrot seeds, citrus peels and seeds, and malt root extracts have been reported for their anti oxidative potentials (Bonnely et al., 2000). In addition to the potential therapeutic effects (Ames et al., 1993), antioxidants have been known to have well defined role as preservatives.

They have been defined by the US food and Drug administration (FDA) as substance used to preserve food by retarding deterioration or discoloration caused by oxidation (Dziezak, 1986). Antioxidants are also referred as chelators, which bind metal ions, such as, copper and iron that catalyse lipid oxidation by oxygen scavengers or those compounds that react with oxygen in closed systems and secondary antioxidants, which function by breaking down the hydroperoxides (Shahidi et al., 1992). The chemical characteristics of antioxidants including their solubility, regenerative activity relationship and bioavailability are important factors when considering their role in human health. The truffles *viz*., *Terfezia* and *Piscoa* spp. and several mushrooms *viz*., *Lepista nuda, Agrocybe cylidracea, Cantharellus tutescens,* and *Hydrum sepandum* exhibit higher percentage of oxidative inhibition based on lipid peroxidation deoxyribose and peroxidase (Murcia et al., 2002). According to the results of this study, it is clearly indicated that calcium carbonate has increased the antioxidant activities. Moreover, the mushroom species can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. This work suggested that calcium carbonate could be used as a suitable additive to enhance the production of antioxidative substances in the mushroom food industry and research field.

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