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Effect of organic additives on enzymatic antioxidants in Lentinus edodes (Berkley).

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Abstract

Lentinus edodes is revered in asian medicine for its health-promoting effects, including antiviral, antifungal, antioxidant, and antitumor effects boosting the immune system, lower in cholesterol, anticoagulant and cancer treatment. It is important nutritionally because of its higher protein, dietary fibers and important mineral contents. The present study investigate the various organic additives viz., rice flour, wheat flour ,corn flour, horse gram flour, sorghum flour, blackgram flour, greengram flour and tapioca flour for the bio mass production and its influence in the enzymatic antioxidants in Lentinus edodes. The result revealed that among the various organic additives, the sorghum flour significantly increased the antioxidative substances compared to other additives.

Introduction

Edible mushrooms have been widely used as human food for centuries and have been appreciated for texture and flavour as well as some medicinal and tonic attributes. However, the awareness of mushrooms as being a healthy food and as an important source of biological active substance with medicinal value has only recently emerged (Koertge *et al*., 2003). In the 21st century, the utilization of natural resources in food and medicine industries has become an international frontier due to their low toxicity and high specificity to activate immune system in body (Tsuchiya *et al*., 2003). Globally, Lentinus edodes (shiitake) is the second most popular edible mushroom, its importance being attributed to both its nutritional value and medical applications (Hatvani, 2001).

Materials and methods

Effect of organic additives on enzymatic antioxidants in *Lentinus edodes.*

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Various organic additives *viz.,* rice flour, wheat flour ,corn flour, horse gram flour, sorghum flour, blackgram flour, greengram flour and tapioca flour were added, separately in cazpek's broth at one and two per cent conc. levels (Kalaiselvan, 2007). The amended media (50 ml) were dispensed with additives in 100 ml Erlenmeyer flask and sterilized at 15 psi for 1h. After cooling, the flasks were inoculated with a disc (9 mm dia.) obtained from the peripheral growth of 11days old culture of *L.edodes* and incubated at 25±2^{0}C for 15 days. Each treatment is replicated thrice and the broth without amendment served as control. After extracting supernatant from the individual treatment flask, the mycelia growth retained in Whatman No.1 filter paper was weighed and recorded as fresh weight of the mycelia. The fresh mycelia were then oven dried separately, at 80ºC for 6 h. and the mycelial dry weight (biomass) was determined and recorded.

Preparation and extraction of the sample

In the culture filtrate, the mycelial mat from the individual treatment retained in Whatman No.1 filter paper was used for the analysis of enzymatic antioxidants. From each sample 5g was transferred into a beaker and methanol was added in the ratio of 1:10 and stirred for one hour. The extract was left overnight. The extract was separated from the residue by filtration through Whatman No.1 filter paper. The residue was re-extracted twice and the two extracts combined. The sample was lyophilized and the residual solvent extract was removed under reduced pressure at 40^{0}C using a rotary evaporator. Extracts were produced in triplicates and used for the assay of antioxidant activity (Anil, 2006).

Biochemical analysis

The mushroom samples were prepared in 0.1 M phosphate buffer and used for biochemical analysis. The extracts were assayed for enzymatic antioxidants such as catalase, superoxide dismutase, glutathione peroxidase, peroxidase, glutathione-S-transferase and glutathione reductase .

Estimation of Catalase (Sinha, 1972)

The assay mixture contained 0.5 ml of H_2O_2, 1.0 ml of 0.01 M Phosphate buffer (pH 7.0) and 0.4 ml water, to which 0.2 ml of the enzyme was added to initiate the reaction. Two ml of the dichromate / acetic acid reagent was added after 0, 30, 60 and 90 seconds of incubation. To

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the control tube the enzyme was added after the addition of the acid reagent. The tubes were then heated for ten min and the colour developed was read at 610 nm. The activity of catalase was expressed as µ mole of H_2O_2 decomposed / min. / mg protein.

Estimation of Superoxide Dismutase (Chao, 2001)

Pipetted out 1.4 ml aliquot of the reaction mixture in a test tube to which 100 µl of the enzyme extract was added followed by a pre incubation at 37^0C for five min. 80 µl of riboflavin was added and the tubes were exposed for ten min. to 200 W Philips fluorescent lamps. The control tube contained equal amount of buffer instead of sample. The sample and its respective control were run together. At the end of the exposure time, one ml of Greiss reagent was added to each tube and the absorbance of the colour formed was measured at 543 nm. The enzyme activity was expressed as inhibition of 50 per cent nitrite formation / min. / mg protein.

Estimation of Glutathione Peroxidase (Rotruck *et al.*, 1973)

Pipetted out 0.4 ml of 0.4 M sodium phosphate buffer (Ph 7.0), 0.1 ml of sodium azide, 0.2 ml of reduced glutathione, 0.1 ml of H_2O_2, 0.2 ml of enzyme extract and 1.0 ml of water were added to makeup the final volume of 2.0 ml. The tubes were incubated for 0, 30, 60 and 90 seconds. The reaction was then terminated by the addition of 0.5 ml TCA. To determine the glutathione content, two ml of the supernatant was removed by centrifugation and added to three ml disodium hydrogen phosphate solution and one ml of DTNB reagent. The colour developed was read at 412 nm. Standards in the range of 200-1000 µg were taken and treated in the similar manner. The activity was expressed in terms of µg of glutathione utilized / min./ mg protein.

Estimation of Peroxidase (Sadasivam and Manickam, 1972)

Pipetted out three ml of 0.05 M pyrogallol solution and 0.1 ml of enzyme extract in a test tube and the spectrometer was adjusted to read 0 at 430 nm. Added 0.5 ml of one per cent H_2O_2 in the test cuvette, mixed and recorded the change in absorbance for every 30 seconds up to three min. The difference in OD change per min. with and without enzyme addition was a measure of peroxidase activity. The activity was expressed in terms of µ moles of pyrogallol oxidized / min. / mg protein.

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Estimation of Glutathione-S-Transferase (Lin, 1999)

Pipetted out one ml of 0.5 M Phosphate buffer, 0.1 ml of enzyme extract to which 1.7 ml of water and 0.1 ml of CDNB were added and incubated at 37^0C for five min. After incubation, 0.1 ml of reduced glutathione was added. The increase in optical density of the enzyme was measured against that of the blank at 340 nm. The enzyme activity was calculated in terms of µ moles of CDNB conjugate formed / min. / mg protein.

Estimation of Glutathione Reductase (Lin, 1999)

Pipetted out 0.2 ml of enzyme extract, 1.5 ml of 0.3 M Phosphate buffer, 0.5 ml of EDTA, 0.2 ml of GSSG and 0.1 ml NADPH was added. The decrease in optical density of the enzyme was measured against that of the blank at 340 nm. The enzyme activity was calculated in terms of µ moles of glutathione utilized / min / mg protein.

Result and Discussion

Effect of organic additives on enzymatic antioxidative substances in *Lentinus edodes*

The results (Table 1and 2) of the experiment clearly indicated that among the various organic additives, the sorghum flour significantly increased the antioxidative substances compared to other additives. The additive sorghum flour recorded maximum amount of enzymatic antioxidant *viz.,* catalase (7.55), superoxide dismutase (5.76), glutathione peroxidase (23.98), peroxidase (12.23), glutathione-S-transferase (37.00) and glutathione reductase (12.09), compared to other additives. This result corroborates with the findings of Kalaiselvan, (2007), who reported increased level of enzymatic and non-enzymatic antioxidative substances due to addition of sorghum flour in *Volvariella volvacea*. Similarly, The antioxidative substances of shiitake mushroom has also been reported by several workers (Yang *et al.,* 2002a; 2002b; Cheung *et al.,* 2003; Anil, 2006; Choi *et al.,* 2006; Kitzberger *et al.,* 2007; Yen *et al.,* 2007). Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases (Fuhrman *et al.,* 1995), cancers (Dragsted *et al.,* 1993; Renaud *et al.,* 1999), neurogenerative diseases (Joseph *et al.,* 1999), inflammation (Lietty *et al.,* 1976) and problems caused by cell and cutaneous ageing (Prior and Coa, 2000). Thus, the higher levels of enzymatic antioxidative substances observed in the present study with *L. edodes* makes it an ideal food supplement for human beings for a better and healthy living.

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Table : 1 Effect of organic additives on biomass production of *L.edodes*

Tr. no	Additives	Mycelial fresh wt. (g)		Mycelial dry wt. (g)	
		1%	2%	1%	2%
1	Rice flour	4.31 f	6.25 f	1.38 f	1.56 f
2	Wheat flour	4.91 e	6.74 e	1.47 e	1.62 e
3	Tapioca flour	5.75 c	7.93 c	1.65 c	1.91 c
4	Horse gram flour	5.23 d	7.21 d	1.58 d	1.85d
5	Sorghum flour	6.74 a	8.92 a	1.86 a	2.12 a
6	Black gram flour	3.72 g	5.81 g	1.29 d	1.32 h
7	Green gram flour	3.95 g	5.23 h	1.32 h	1.48 g
8	Corn flour	6.25 b	8.21 b	1.73 h	2.03b
9	control	2.51 i		1.26 i	

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Table 2: Effect of organic additives on enzymatic antioxidative substances in *L.edodes*

Sl.No.	Additives (2 %)	Catalase	Superoxide dismutase	Glutathione peroxidase	Peroxidase	Glutathione -S-transferase	Glutathione reductase
1	Rice flour	6.96$_{d}$	5.16$_{e}$	22.28$_{c}$	10.37$_{f}$	35.44$_{e}$	14.67$_{c}$
2	Wheat flour	7.04$_{c}$	5.20$_{d}$	22.32$_{c}$	10.65$_{e}$	35.44$_{e}$	14.75$_{b}$
3	Tapioca flour	7.19$_{b}$	5.35$_{c}$	22.55$_{b}$	11.07$_{c}$	35.99$_{c}$	14.93$_{b}$
4	Horse gram flour	7.10$_{c}$	5.25$_{d}$	22.44$_{c}$	10.98$_{d}$	35.95$_{d}$	14.88$_{b}$
5	Sorghum flour	7.55$_{a}$	5.76$_{a}$	23.98$_{a}$	12.23$_{a}$	37.00$_{a}$	15.09$_{a}$
6	Black gram flour	6.85$_{d}$	5.09$_{f}$	22.15$_{d}$	10.14$_{h}$	35.13$_{g}$	14.44$_{d}$
7	Green gram flour	6.87$_{d}$	5.14$_{e}$	22.20$_{c}$	10.23$_{g}$	35.23$_{f}$	14.55$_{c}$
8	Corn flour	7.22$_{b}$	5.44$_{b}$	22.62$_{b}$	11.97$_{b}$	36.68$_{b}$	14.97$_{b}$
9	control	6.14$_{e}$	4.98$_{g}$	21.96$_{e}$	10.01$_{h}$	35.05$_{g}$	12.07$_{e}$

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