International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

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

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Abstract

Shiitake (*Lentinus edodes*) is globally a well known cultivated species, but yet to find a place in Indian markets. Lack of cultivation technology on locally available substrates and suitable high temperature strains are the reason for its non-availability in India. 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 Non 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

Mushrooms are rich in dietary fiber, minerals and vitamins and low in fat (Manzi et al., 2001). The consumption of mushrooms or compounds present in mushroom extracts is suggested to have several health benefits. The crude protein content (chitin-N removed) of cultivated mushrooms is generally high (20–44% of dry matter) but the fat content is low (3–7% of dry matter) (Dikeman et al., 2005).They have recently become attractive as food (physiologically functional) and as sources for the development of drugs (Manzi and Pizzoferrato, 2000). Medicinal mushroom extracts have been considered as important remedies for the prevention and treatment of many diseases for thousands of years (Smith et al., 2002; Wasser, 2002).

Materials and methods

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

Various organic additives *viz.,* rice flour, wheat flour , horse gram flour, sorghum 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

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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 non-enzymatic antioxidants such as total glutathione, vitamin A, vitamin C, vitamin E and total carotenoids.

Estimation of Total Glutathione (Mori *et al.*, 1989)

The enzyme extract (0.5 ml) was mixed with 0.5 ml of 5 per cent TCA. The precipitated protein was centrifuged at 1000 rpm for ten min. 0.1 ml of the supernatant was made up to one ml with 1.0 ml sodium phosphate buffer (pH 8.0) and 2.0 ml of freshly prepared DTNB were added. The absorbance was read after 10 min. at 412 nm against a reagent blank. A set of standards were also treated in the above manner. The amount of glutathione was expressed as µg / mg protein.

Estimation of vitamin A (Nield and Ashwood, 1963)

Pipetted out one ml of ten per cent homogenate, one ml of saponification mixture (2N/KOH in 90% alcohol) was added and heated for 20 min. at 60^{0}C. Twenty five ml of water was added to the mixture after cooling to room temp. and the solution was transferred to a separating funnel. It was then extracted thrice with using 10, 15 and 25 ml of petroleum ether (40-60^{0}C). The ether extracts were pooled and washed with 50-100 ml of dist. water repeatedly until the wash was free of alkali.

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The petroleum ether extract was then dried by adding anhydrous sodium sulphate. The volume of the extract was noted. Three ml of petroleum ether was transferred to a cuvette and read at 420 nm against a petroleum ether blank without delay to prevent evaporation of the solvent and destruction of carotenoids by light. Marked this reading as A_1. The β-carotene working standards were measured at 450 nm.The aliquots were evaporated to dryness at 60^0C in a water bath. The residue was taken immediately and two ml TFA reagent were added to it. The mixture was rapidly transferred to a cuvette and the absorbance was measured at 620 nm exactly after the addition of TFA reagent. Marked this reading as A_2. The vitamin A working standard was read at 620 nm.

Calculation

The accurate calculation of the vitamin A content, it was necessary to correct for the absorbance contributed by carotene at 620 nm.

$A_3 = A_2 - A_1$

A_1 = Absorbance of carotene at 450 nm

A_2 = Absorbance at 620 nm due to both carotene and vitamin A.

A_3 = Absorbance at 620 nm of vitamin A.

$$\text{Sample} = \frac{A_3 \times \mu g \text{ retinol calibarator / cuvette} \times 3 \times \text{total volume}}{A_{620} \text{ retinol calibarator} \times 2 \times \text{gram}}$$

3 = Volume of petroleum ether from 1.0 ml extract

2 = Aliquot of the petroleum ether used for the assay

1 = 10 per cent extract taken from initial sample

The results were expressed as µg / g tissue.

Estimation of vitamin C (Sumathi, 1998)

Pipetted out one ml of enzyme extract and made up the volume to three ml by adding dist. water. Added one ml of dinitro phenyl hydrazine reagent followed by one to two drops of thiourea into each tube. A blank was set as above but with water in place of ascorbic acid solution. Mixed the contents of the tube thoroughly and incubated at 37^0C for three hours. After incubation the tubes were kept in ice bath. Dissolved the orange red azazone crystals formed by adding seven ml of 80 per cent sulphuric acid drop wise while the tubes were still in water bath. The tubes in the ice bath were removed and allowed to stand for 30 min. at room temp. and measured the absorbance at 540 nm. The results were expressed as µg / g tissue.

Estimation of vitamin E (Mattila *et al.*, 2001)

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Into three stoppered centrifuge tubes (test, standard and blank) pipetted out 1.5 ml of each tissue extract, 1.5 ml of the standard and 1.5 ml of water, respectively. To the treatment and blank added 1.5 ml ethanol and to the standard added 1.5 ml of water. Added 1.5 ml of xylene to all the tubes, stoppered, mixed well and centrifuged. Transferred one ml of xylene layer into another stoppered tube, taking care not to include any ethanol or protein. Added one ml of 2,2-dipyridyl reagent to each tube, stoppered and mixed. Pipetted out 1.5 ml of the mixtures into spectrophotometer cuvettes and read the absorbance of test and standard against the blank at 460 nm. Then in turn beginning with the blank, added 0.33 ml of ferric chloride solution. Mixed well and after exactly 1.5 minutes read test and standard against the blank at 520 nm. The results were expressed as µg/g tissue. The amount of vitamin E can be calculated using the formula

$$\text{Vitamin E } (\mu g / g) = \frac{(\Delta A_{520\ nm} - \Delta A_{450\ nm} \times \text{conc [S]} \times 0.29 \times \text{Total volume})}{\Delta A_{520\ nm} \times \text{Volume for experiment} \times \text{Weight of sample}}$$

Estimation of Total Carotenoids (Gerester, 1997)

Weighed five to ten g of the sample. Saponified for about thirty min. in a shaking water bath at 37^0C after extracting the alcoholic KOH. Transferred the saponified extract in to a separating funnel (packed with glass wool and calcium carbonate) containing ten to fifteen ml of petroleum ether and mixed gently. The carotenoid pigment was taken up into the petroleum ether layer. The lower aqueous phase was transferred to another separating funnel and the petroleum ether extract containing the carotenoid pigments to an amber coloured bottle. Repeated the extraction of the aqueous phase similarly with petroleum ether until it is colourless and discarded the aqueous portion. To the petroleum ether extract added a small quantity of sodium sulphate to remove turbidity. Noted the final volume of the petroleum ether extract and diluted if needed by a known dilution factor. The absorbance at 450 and 503 nm was noted in a spectrophotometer using petroleum ether as a blank. The results were expressed as µg / g tissue.

Calculation

$$\text{Carotenoids } (\mu g) = \frac{P \times 4 \times V \times 100}{W}$$

P = Optical density of the sample
V= Volume of the sample
W= Weight of the sample

Result and Discussion

Effect of organic additives on non enzymatic antioxidative substances in L. edodes

The results (Table 1 and 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 non-enzymatic antioxidative substances viz., total glutathione (14.84), vitamin A (1.90), vitamin C (0.41), vitamin E (0.13) and total carotenoids (16.00) were also found maximum in medium amended with sorghum flour.

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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). Thus, the higher levels of enzymatic and non-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	3.31 f	5.25 f	1.31 f	1.56 f
2	Wheat flour	4.93 e	6.75 e	1.76 e	1.92 e
3	Tapioca flour	5.65 c	7.13 c	1.56 c	1.98 c
4	Horse gram flour	6.21 d	7.27 d	1.82 d	1.91d
5	Sorghum flour	7.72 a	8.95 a	1.91 a	3.21 a
6	control	2.51 i		1.26 i	

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

Sl.No.	Additives (2 %)	Total Glutathione	Vitamin A	Vitamin C	Vitamin E	Total Carotenoids
1	Rice flour	13.09$_{e}$	1.10$_{f}$	0.21$_{e}$	0.05$_{f}$	15.40$_{f}$
2	Wheat flour	13.40$_{d}$	1.28$_{c}$	0.22$_{e}$	0.06$_{e}$	15.51$_{d}$
3	Tapioca flour	13.85$_{b}$	1.56$_{b}$	0.31$_{c}$	0.10$_{c}$	15.85$_{b}$
4	Horse gram flour	13.71$_{c}$	1.49$_{c}$	0.27$_{d}$	0.07$_{d}$	15.72$_{c}$
5	Sorghum flour	14.84$_{a}$	1.90$_{a}$	0.41$_{a}$	0.13$_{a}$	16.00$_{a}$
6	Black gram flour	12.95$_{f}$	1.02$_{g}$	0.16$_{f}$	0.03$_{h}$	15.31$_{g}$
7	Green gram flour	13.00$_{e}$	1.06$_{g}$	0.18$_{f}$	0.04$_{g}$	15.36$_{f}$
8	Corn flour	13.94$_{b}$	1.58$_{b}$	0.36$_{b}$	0.11$_{b}$	15.88$_{b}$
9	control	12.54$_{g}$	0.82$_{h}$	0.09$_{g}$	0.03$_{h}$	14.22$_{h}$

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