Int.J.Curr.Microbiol.App.Sci (2019) *8*(2): 3031-3036

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 02 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.802.355

Antioxidant Properties of Paddy Straw Mushroom [*Volvariella volvacea* (Bull. ex Fr.)] Sing

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Keywords

Volvariella volvacea, Antioxidant properties, Additives

Article Info

Accepted:
20 January 2019
Available Online:
10 February 2019

A B S T R A C T

Paddy straw mushroom/ Chinese mushroom (*Volvariella volvacea*) is highly suited in tropical climates. The success of this mushroom production is solely depended on high quality spawn and improved technologies. Among the additives, calcium carbonate amended beds recorded maximum amount of total glutathione, total phenol, flavonoids, lycopene, total carotenoids, vitamin A and vitamin C were recorded. This was followed by horse gram, gypsum and calcium carbonate + gypsum. In non-amended medium significantly very less amount of antioxidative substances were recorded.

Introduction

The genus *Volvariella* (paddy straw mushroom) comprised a group of several species, which can be found growing on a variety of substrates in tropical and sub-tropical regions. *V. volvacea* (Bull. ex Fr.) Sing., is probably the best known species, as it has been traditionally cultivated in Southeast Asia since the 18th century (Chang, 1977). At present time, *V. volvacea* is the third most important cultivated mushroom reaching total world production of 287 metric tones (Chang and Miles, 1993). In India, Su and Seth (1940) have first cultivated straw mushroom but the scientific cultivation using spawn was successfully demonstrated by Thomas *et al.*, (1943). It is commonly known as Chinese mushroom, the most favourite mushroom in South Asian countries because of its excellent delicacy, high protein, aminoacid, vitamins and mineral contents (Thakur and Yadav, 2006). The climatic conditions prevailing in the Indian plains seem to be quite suitable for large scale production of paddy straw mushroom. In recent years, the use of some synthetic antioxidants has been restricted because of

their possible toxic and carcinogenic effects (Gazzani *et al.,* 1998 and Frankel *et al.,* 1985). This concern has resulted in an increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties (Duh *et al.,* 1992) foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases (Dragsted *et al.,* 1993), neurodegenerative diseases, Parkinson's and Ahlzeimer's diseases (Clarke, 1999 and Joseph *et al.,* 1999) inflammation and problems caused by cell and cutaneous aging (Ames *et al.,* 1993; Gaulejac *et al.,* 1999 Prior and Coa, 2000). Thus the natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects (Ames *et al.,* 1993).in view of this fact, the present study has been programmed to estimate the non-enzymatic antioxidative substances due to additives in *V.volvacea.*

Materials and Methods

Preparation of mushroom bed

Paddy straw was soaked overnight in cleaned water and stream sterilized at 15 lbs pressure for 30 min. Excess water was drained after steam sterilization and shade dried to have 65-75 per cent moisture content. The substrates was made into small twists of 2.5 m long and 5-8 cm dia. weighing about 1.25 kg (dry weight). Totally four twisted bundles were used for the preparation of circular compact beds. Initially, the twists were made clockwise in a circular fashion. The mycelial discs of 8 mm dia. were taken from the seven day old actively growing culture and inoculated @ three disc per layer. On the surface layer s various organic and inorganic additives as listed earlier were sprinkled (2 per cent). Likewise totally four twisted bundles were used. The completed beds were pressed tightly on the wooden rack in a polyhouse with sufficient temp (30-35ºC) and humidity (80-85) per cent was maintained. The beds were regularly sprayed with water. The harvested mushrooms were used for assay of antioxidative substances. Non amended additive used as control. Straw bed without additive served as control.

Collection and drying of mushrooms

Harvested mushrooms were sun-dried separately for 24 h and dried in an oven at 60^{o}c for 30 min. Then, the mushrooms were powdered using pulverizer.

Estimation of non enzymatic antioxidative substances

Mushroom extracts were assayed for non-enzymatic antioxidants such as total glutathione, total phenols, flavanoides, lycopene, total carotenoids, vitamin A, and vitamin C.

Preparation and extraction of samples

Five gram of samples from each treatment samples was extracted by stirring with 100ml of methanol and filtered through Whatmann No. 4 filter paper. The residue was reextracted twice. The sample was lyophilized and residual solvent extract was removed under reduced pressure at 4^{0}c using a rotary evaporator. Extracts were produced in triplicates and used for the analysis of antioxidative substances.

Estimation of total reduced glutathione (Mori *et al.,* 1989)

Mushroom extract 0.5 ml was mixed with 0.5 ml of 5% TCA. The precipitated protein was centrifuged down at 1000 rpm for 10 minutes. 0.1 ml of the supernatant was made upto 1.0

ml with sodium phosphate buffer. 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 total phenols (Sumathi, 1998)

Pipetted out 0.1 ml of sample into test tubes and the volume was made to 3.0 ml with distilled water. 0.5 ml of Folin-Ciocalteau reagent was added. After 3 minutes, 2.0 ml of 20 % sodium carbonate was added. Mixed thoroughly, and placed in boiling water bath for exactly one minute, cooled and read the absorbance at 650 nm against a reagent blank. A set of standards were also treated in the above manner. The amount of phenols was expressed as mg /mg tissue.

Estimation of flavanoids (Hertog *et al.*, 1992)

Added 0.5 ml of the sample to a test tube containing 1.25 ml of distilled water. Then added 0.075 ml of 5% sodium nitrite solution and allowed to stand for 5 min. Added 0.15 ml of 10% aluminium chloride, after 6 min 0.5 ml of 1 M sodium hydroxide was added and the mixture was diluted with another 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately. The flavanoid content was expressed as milligram catechin equivalents /g sample.

Estimation of total lycopene and carotenoids (Gerster, 1997)

Weighed 5 to 10 g of the sample. Saponified for about 30 minutes in a shaking water bath at 37^0c after extracting the alcoholic KOH. Transferred the saponified extract into a separating funnel (packed with glass wool and calcium carbonate) containing 10 to 15 ml of petroleum ether and mixed gently. Taken up the carotenoid pigments into the petroleum ether layer. Transferred the lower aqueous phase 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. Discarded the aqueous. 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 nm and 503 nm was noted in a spectrophotometer using petroleum ether as a blank.

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

(Lycopene mg/100g) =

$$\frac{3.1206 \times \text{OD sample} \times \text{vol made up} \times \text{dilution} \times 100}{1\text{x weight of the sample} \times 1000}$$

Estimation of vitamin A (Nield *et al.*, 1963)

To 1.0 ml of 10 per cent homogenate 1.0 ml of saponification mixture (2N/KOH in alcohol) was added and heated under gentle reflux for 20 min at 60^0C. Twenty five ml of water was added to the mixture after cooling to room temperature and the solution was transferred to a separating funnel. It was then extracted thrice with using 25, 15 and 10 ml of petroleum ether (40-60^0C). The ether extracts were pooled and washed with 50-100 ml of distilled water repeatedly until the wash water was free of alkali. The petroleum ether

extract was then dried by adding anhydrous sodium sulphate. The volume of the extract was noted. 3.0 ml of petroleum ether phase 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 and marked this reading as A1. The β–carotene working standards were measured at 450 nm.

The aliquots were evaporated to dry ness at 60^0c in a water bath. The residue was taken immediately and 2.0 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 and marked this reading as A2. The vitamin A working standard was read at 620 nm.

Calculation

For 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.

Sample =

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

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)

One ml of brominated sample extract was taken and made up the volume to 3.0 ml by adding distilled water. Added 1.0 ml of dinitro phenyl hydrazine reagent followed by 1 to 2 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 3 hours. After incubation the tubes were kept in the ice bath. Dissolved the orange red azazone crystals formed by adding 7.0 ml of 80 per cent sulphuric acid drop wise while the tubes were still in the water bath. The tubes in the ice bath were removed and allowed to stand for 30 minutes at room temperature and measured the absorbance at 540 nm. The result is expressed as µg/g tissue.

Results and Discussion

The effect of various organic and inorganic additives on the production of non enzymatic antioxidative substances *viz.*, total glutathione, total phenol, flavonoids, lycopene, total carotenoids, vitamin A, and vitamin C were presented in Table 1.

Among the additives, calcium carbonate amended beds recorded maximum amount of total glutathione (395.25 µg/ g), total phenol (16.72 mg/g), flavonoids (9.17 mg/g), lycopene (1.99 mg/g), total carotenoids (0.35 µg/g), vitamin A (6.23 µg/g) and vitamin C (0.54 mg/g). This was followed by horse gram (342.50 µg/g; 12.05 mg/g; 8.00 mg/g; 1.73 mg/g; 0.20 µg/g; 4.52 µg/g and 0.43 mg/g), gypsum (210.26 µg/g; 13.52 mg/g; 7.40 mg/g; 1.70 mg/g; 0.19 µg/g; 4.05 µg/g and 0.42 mg/g) and calcium carbonate + gypsum (210.0 µg/g; 12.68 mg/g; 7.05 mg/g; 1.75 mg/g; 0.18 µg/g; 3.90 µg/g and 0.41mg/g). In non-amended medium significantly very less amount of antioxidative substances were recorded.

Traditionally *V. volvacea* has been grown on rice straw. That tradition earned it the name of paddy straw mushroom (Chang, 1983). The straw mushroom cultivation in tropical/subtropical areas is still less advanced. The climatic condition prevailing in the Indian plains seems to be quit suitable for large scale production. Practically in India a very little improvement in the cultivation technique has been done during the last two decades. Major drawback in the cultivation of *Volvariella* is the very low biological efficiency (B.E) as compared to other tropical mushrooms. If culture technique improved, it should be cultivated widely and cheaply as other common vegetables which can be consumed regularly by all people. In view of the above facts, the present thesis research was aimed to increase the biological efficiency by utilizing various new cultivation technologies for yield improvement. The results obtained were discussed herein.

Antioxidative substances

Foods rich in antioxidative substances have been showed to play essential role in the prevention of cardiovascular diseases (Dragsted *et al.*, 1993), cencers (Dragsted *et al.*, 1993), neurogenerative diseases (Joseph *et al.*, 1999), inflammation (Joseph *et al.*, 1999) and cutaneous ageing (Prior and Coa, 2000), The use of synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects (Gazzani *et al.*, 1998). The present study was therefore aimed to study the natural antioxidative (non-enzymatic) substances *viz.*, total glutothione, total phenol, flavanoides, lycopene, total carotenoids, vitamin A and vitamini C. Among the additives, calcium carbonate recorded maximum amount of glutothione
(395.25 µg/g, total phenol (16.27 mg/g), flavanoides (9.17 mg/g), lycopene (1.99 mg/g) total carotenoide (0.35 µg/g), and vitamin A (6.23 µg/g) and vitamin C (0.54 mg/g) and this was followed by horse gram (342.50 µg/g; 12.05 mg/g; 8.00 mg/g; 1.73 mg/g; 0.20 µg/g; 4.52 µg/g and 0.43 mg/g). Murcia *et al.*, (2002) reported that all truffles (*Terfezia* and *Piscoa* spp.) and five mushrooms *(Leptista nuda, Lentinus edodes, Agrocybe cylindracea, Cantharellus lutescens and Hydrum sepundum*) exihibited higher per cent of oxidative inhibition based on lipid peroxidation, deoxyribose and peroxidase. Methanol extract of *P. florida* have potent hydroxyl radical scavenging and lipid peroxidation inhibition (antioxidant) activities. The antioxidant activities in *Ganoderma lucidum, P. florida, P. sajor-caju*, as scavenging activity was demonstrated by Lakshmi *et al.,* (2005). Recently Prabhakara (2006) reported high level of antioxidative substances due to calcium carbonate activity in *P.euos.*

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How to cite this article:

Sudha, A., P. Geetha and Rajesh, M. 2019. Antioxidant Properties of Paddy Straw Mushroom (*Volvariella volvacea* (Bull. ex Fr.)) Sing. *Int.J.Curr.Microbiol.App.Sci.* 8(02): 3031-3036.
doi: https://doi.org/10.20546/ijcmas.2019.802.355