

Antioxidative Substances (Non enzymatic) in Oyster Mushrooms (*Pleurotus* spp.)

A. Sudha*, S. Srividhya, P. Geetha and M. Vijayakumar

Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Sandhiyur, -636203, T.N., India

*Corresponding author

ABSTRACT

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Among the oysters, *P. eous* recorded highest levels of non-enzymatic antioxidative substances. Total glutathione content was significantly very high in *P. eous*, followed by *P. sajor-caju*, *P. flabellatus* and *H. ulmarius*. High level of total phenols, flavanoids, lycopene total carotenoid and vitamin A were recorded in *P. eous*. This was followed by *P. sajor-caju*, *P. flabellatus* and *H. ulmarius*. There was no significant difference in vitamin C content among the oyster mushrooms.

Introduction

Mushrooms possess high contents of qualitative protein, crude fibre, minerals and vitamins. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. They produce a wide range of secondary metabolites with high therapeutic value. Health promoting properties, e.g. antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects, have been reported for some species of mushrooms. Both fruiting bodies and the mycelium contain compounds with wide ranging antioxidant and antimicrobial activities. Protein rich (*Pleurotus* spp.) delicious food will help to overcome protein

deficiency in the diet of Indian population (Madhusudhanan and Chandra Mohan, 2000). Malnutrition is one of the major health problems in developing countries. Cereal based Indian diet suffers from inadequate protein content. Oyster mushrooms are therefore recognized as non-conventional source of protein, which can bridge the protein gap in the Indian diet. Mushrooms are essential supplements to the cereal diet (Vijaya Khader *et al.*, 1999). The nutritional value of mushroom is rated as one in between meat and vegetables. Oyster mushrooms are rich in amino acids, vitamin, protein, minerals and fiber but low in fat. It is an ideal food for patients suffering from heart ailments, diabetic and ulcers (Mohan and Subash

Chandra Bose, 1999). They are reported to possess antigastric ulcer activities, reduced the rate of nephron deterioration which might extend the life span of chronic renal failure patient and controls constipation due to high fiber content (Anon, 2000).

Besides nutritive value oyster mushrooms have immense potential as medicines too. In recent years, mushrooms have been exploited for their antioxidative potentials (Ajith and Janardhanan, 2001; Murcia *et al.*, 2002 and Lakshmi *et al.*, 2005). Foods rich in antioxidant have been shown to play an essential role in the prevention of cardiovascular diseases, neurodegenerative diseases and inflammation.

Commonly grown oyster mushroom fungi *viz.*, *Pleurotus eous* (Berk.) Sacc, *Pleurotus Sajor-caju* (Fr.) Singer, *Pleurotus flabellatus* (Berk and Br. Sacc) and *Hypsizygus ulmarius* (Bulliard: Fries) were used for the present study.

Materials and Methods

Estimation of non-enzymatic antioxidative substances

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

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

Mushroom extract 0.5 ml was mixed with 0.5 ml of 5% TCA. The precipitate d 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 1042 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 $\mu\text{g}/\text{mg}$ protein.

Estimation of total phenols (Sumathi, 1998)

Pipetted out 0.1 ml of sample into test tubes. Made up the volume in to 3.0 ml with distilled water. Added 0.5 ml of Folin-Ciocalteu reagent. After 3 minutes added 2.0 ml of 20 % sodium carbonate.

Mixed thoroughly, placed the tubes 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 $\mu\text{g}/\text{mg}$ tissue.

Estimation of vitamin A (Nield and Pearson, 1963)

To 1.0 ml of 10% homogenate 1.0 ml of saponification mixture (2N/KOH in alcohol) was added and heated under gentle reflux for 20 min at 60°C. 25 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°C). 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. Marked this reading as A1, the β -carotene working standards were measured at 450 nm.

The aliquots were evaporated to dryness at 60°C 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. 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.

$$A_3 = A_2 - A_1$$

A₁ = Absorbance of carotene at 450 nm

A₂ = Absorbance at 620 nm due to both carotene and vitamin A.

A₃ = Absorbance at 620 nm of vitamin A.

$$A_3 \times \mu\text{g retinol calibrator /cuvette} \times 3 \times \text{total volume}$$

$$\text{Sample} = \frac{\text{-----}}{A_{620} \text{ retinol calibrator} \times 2 \times \text{gram}}$$

3 = Volume of petroleum ether from 1.0 ml extract

2 = Aliquot of the petroleum ether used for the assay

1 = 10% extract taken from initial sample

The results were expressed as $\mu\text{g/g}$ 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°C 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% 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 $\mu\text{g/g}$ tissue.

Estimation of total carotenoids and lycopene (Gerster, 1997)

Weighed 5 to 10 g of the sample. Saponified for about 30 minutes in a shaking water bath at 37°C 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

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

P = Optical density of the sample

V = Volume of the sample

W = Weight of the sample

(Lycopene mg/100g) =

$$\frac{3.1206 \times \text{OD sample} \times \text{vol made up}}{\text{x dilution} \times 100}$$

1x weight of the sample x 1000

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.

Results and Discussion

In oysters, non-enzymatic antioxidative substance *viz.*, total glutathione, total phenol, flavanoids, lycopene, total carotenoids, vitamin A and vitamin C were estimated and presented in table 1. Among the oysters, *P. eous* recorded highest levels of non-enzymatic antioxidative substances. Total glutathione content was significantly very high in *P. eous* (408.37 $\mu\text{m} / \text{g}$), followed by *P. sajor-caju* (352.45 $\mu\text{m} / \text{g}$), *P. flabellatus* (215.80 $\mu\text{m} / \text{g}$) and *H. ulmarius* (187.37 $\mu\text{m} / \text{g}$). High level of total phenols (15.68 mg/g), flavanoids (7.34 mg /g), lycopene (1.96 mg /g), total carotenoid (0.27 $\mu\text{g} / \text{g}$) and vitamin A (5.98 $\mu\text{g} / \text{g}$) were recorded in *P. eous*. This was followed by *P. sajor-caju* (13.52 mg /g; 7.15 mg /g; 1.87 $\mu\text{g} / \text{g}$; 0.20 $\mu\text{g} / \text{g}$; 4.52 $\mu\text{g} / \text{g}$), *P. flabellatus* (10.05 mg /g; 7.05 mg /g; 1.73 mg/g; 0.19 mg /g; 4.05 $\mu\text{g} / \text{g}$) and *H. ulmarius*

(12.72 mg /g; 6.62 mg /g; 1.70mg /g; 0.18 $\mu\text{g} / \text{g}$; 3.90 $\mu\text{g} / \text{g}$). There was no significant difference in vitamin C content (0.41 to 0.46 mg /g) among the oyster mushrooms.

Foods rich in antioxidative substances have been showed to play essential role in the prevention of cardiovascular diseases (Dragsted *et al.*, 1993), cancers (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 investigations was therefore aimed to probe the natural (non-enzymatic) antioxidative substances *viz.*, total glutathione, total phenol, flavanoids, lycopene, total carotenoids, vitamin A and vitamin C, in commonly grown oyster mushrooms.

Among the oysters, *P. eous* recorded high level of natural antioxidative substances (Table 4). Total glutathione content was significantly high in *P. eous* (408.37 $\mu\text{g} / \text{g}$), followed by *P. sajor-caju* (352.45 $\mu\text{g} / \text{g}$), *P. flabellatus* (215.80 $\mu\text{g} / \text{g}$), and *H. ulmarius* (187.37 $\mu\text{g} / \text{g}$). High levels of total phenols (156.68 mg /g), flavanoids (7.34 mg /g), total carotenoid (0.27 $\mu\text{g} / \text{g}$) and Vitamin A (5.98 $\mu\text{g} / \text{g}$) were recorded in *P. eous*.

There was no significant difference in Vitamin C content (0.41-0.46 $\mu\text{g} / \text{g}$) among the oyster mushrooms (Fig.3) Murcia *et al.*, (2002) reported that all truffles (*Terfezia* and *Pisicoa* spp.) and five mushrooms (*Leptista nuda*, *Lentinus edodes*, *Agrocybe cylindracea*, *Cantharellus lutescens* and *Hydrum sepundum*) exhibited higher per cent of oxidative inhibition based on lipid peroxidation, deoxyribose and peroxidase.

Table.1 Non-enzymatic antioxidative substances in oyster mushrooms

SL. NO.	Mushroom	Total glutathione ($\mu\text{m} / \text{g DW}$)	Total phenol (mg /g DW)	Flavanoids (mg /g DW)	Lycopene (mg /g DW)	Total Carotenoids ($\mu\text{g} / \text{DW}$)	Vitamin A ($\mu\text{g} / \text{g DW}$)	Vitamin C (mg /g DW)
1	<i>P. eous</i>	408.37 ^a	15.68 ^a	7.34 ^a	1.96 ^a	0.27 ^a	5.98 ^a	0.46 ^a
2	<i>P. sajor-caju</i>	352.45 ^b	13.52 ^b	7.15 ^b	1.87 ^b	0.20 ^b	4.52 ^b	0.42 ^a
3	<i>P. flabellatus</i>	215.8 ^c	10.05 ^d	7.05 ^{cb}	1.73 ^c	0.19 ^c	4.05 ^c	0.41 ^a
4	<i>H. ulmarius</i>	187.37 ^d	12.72 ^c	6.62 ^d	1.70 ^{dc}	0.18 ^d	3.90 ^d	0.43 ^a

Values are expressed as mean of 3 replicates,

Means followed by a common letter are not significantly different at 5% level by DMRT

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).

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