Effect of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus* (*Oyster mushroom*)

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Abstract

The effect of five different substrates viz. paddy straw, wheat straw, mixture of paddy and wheat straw (in the ratio of 1 : 1), bamboo leaves and lawn grasses on the production of edible *Oyster mushroom* (*Pleurotus ostreatus*) was studied. Wheat straw and a mixture of paddy and wheat straw gave the earliest colonization of fungus. The highest yield of *P. ostreatus* was recorded on wheat straw (29.27 g fresh weight/kg substrate), followed by the combination of paddy and wheat straw (27.96 g fresh weight/kg substrate). Non-enzymatic antioxidant activities were also obtained by estimating vitamins A, C and E. Significant amount of vitamin E was found in both fresh (7.23 mg/g) and dry fruit body (5.93 mg/g) of *P. ostreatus*. All the experiments were carried out in triplicates. [Life Science Journal. 2008; 5(3): 73 – 76] (ISSN: 1097 – 8135).

Keywords: *Pleurotus ostreatus*; substrate; wheat straw; non-enzymatic antioxidant

1 Introduction

Edible mushrooms, or wild edible fungi, have been collected and consumed by people for thousands of years. The archaeological record revealed edible species associated with people living 13000 years ago in Chile (Rojas and Mansur, 1995), but it is in China where the eating of wild fungi is first reliably noted, several hundred years before the birth of Christ (Aaronson, 2000). Edible fungi were collected from forests in ancient Greek and Roman times and highly valued, though more by high-ranking people than by peasants (Buller, 1914).

Edible mushrooms like Pleurotus are known to be among the largest of fungi (Onuoha, 2007). *Pleurotus ostreatus* (*Oyster fungus*) (*P. ostreatus)* is an edible mushroom having excellent flavour and taste (Shah *et al*, 2004). It belongs to class Basidiomycetes, subclass Hollobasidiomycetidae, order Agaricales. It grows wild in the forests of hilly areas and is cultivated in temperate and subtropical regions of the world (Ibekwe *et al*, 2008). Mushroom cultivation technique is spreading nowadays through out the world as these are easy to grow and no any skilled person is required. The *Oyster mushroom* has a high nutritional value due to its high level of vitamins and proteins and its non-saturated fatty acids. Originally cultivated in Asia, it is now cultivated world wide for food. Apart from using *Oyster mushrooms* as a food, the oyster mushroom is produced industrially for uses such as the manufacture of paper pulp, cosmetics or in the pharmaceutical industry. *Oyster mushrooms* can also be used industrially for mycoremediation purposes (Stamet, 1993). Mushrooms are reported to be easily grown on different lignocelluloses wastes such as banana leaves, cereal straw, paper wastes, sawdust and poultry droppings (Fasidi and Kadiri, 1993; Onuoha, 2007; Shah *et al*, 2004).

In the present study the effects of different substrates have been examined on the production of *P. ostreatus*. Non-enzymatic antioxidant activities were also obtained by estimating vitamin A, C and E in this study.

2 Materials and Methods

2.1 Fungal culture, growth conditions and spawn

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production

P. ostreatus was used throughout this study. It was maintained on Potato Dextrose Agar (Himedia, Mumbai, India) plate at 4 °C and was subcultured every four weeks. Fully grown culture of *P. ostreatus* was used for spawn production in a wide mouth bottle using wheat grains. Wheat grains were sterilized first then mixed thoroughly with 1% and 2% of calcium carbonate and calcium sulphate respectively by the grain weight under dry condition. The mixture was filled in a wide mouthed bottle, plugged tightly and sterilized for two consecutive days at 15 psi, 121 °C for 90 min and inoculated with *P. ostreatus* culture. Bottle was incubated at 25 °C for 15 days under dark condition to give an optimum time for spawn production.

2.2 Growth substrates

Five different substrates were selected-paddy straw, wheat straw, mixture of paddy and wheat straw, bamboo leaves and lawn grasses. They were selected because they are readily and locally available.

2.3 Preparation of the substrates for cultivation

All the five substrates (paddy straw, wheat straw, paddy straw 50% + wheat straw 50%, bamboo leaves and lawn grasses) were soaked in water overnight and drained, resulting in a moisture content of approximately 70%. All the substrates were chopped before use. Urea and gypsum were mixed at the rate of 2% and 3% respectively (on dry weight basis). Ingredients were thoroughly mixed by hand. Each substrate was filled in autoclavable plastic bag and autoclaved at 15 psi, 121 °C for 90 min. Sterilized substrate bags were inoculated with grain spawn at a rate of 6% (w/w), heat sealed, and transferred to the incubation room for spawn run. Seed spawn was mixed with substrate layer wise. The pinholes were also made in the bags manually for exhaust of gases. The bags were incubated at 20 ºC under dark condition. The humidity of bags was accomplished by spraying of water on them twice a day. Initial weights of substrate-filled bags were recorded prior to incubation.

2.4 Fruiting

After incubation of 20 days, bags were transported to the fruiting site. Colonization of mycelia was seen clearly through naked eyes in these bags. Bags were cutoff and hanged the whole colonized mycelia with substrate using thread to initiate primordial formation. It was found to be highly compacted. The misting system was set to operate for 2 min every hour throughout the day and night. All bags remained at the fruiting site for 8 – 10 weeks under dark condition at 20 ºC. All the treatments with substrates were performed in triplicates.

2.5 Yield and fruit body analysis

The yield of the *P. ostreatus* on the different substrates was determined by the number and size of the fruit bodies produced. The fruit bodies were harvested at the end of the experiment and number of fruit bodies, diameter, fresh and dry weight of fruit bodies were measured using conventional technique.

2.6 Estimation of vitamins

Fruit bodies grown using wheat straw were collected for the estimation of vitamins. Both fresh and dry fruit bodies were used to estimate the vitamins. Fruit bodies were dried at 70 ºC and powdered before estimation of vitamins. Vitamin A (retinol) was estimated according to the method of Bayfield and Cole (1980). Fruit body sample was extracted with petroleum ether and washing was done using water and layers were separated using separating funnels. Sodium sulfate was added to remove the moisture. To estimate the vitamin A, dried residue was dissolved in chloroform.

Vitamin C (ascorbic acid) was estimated according to the method of Roe and Keuther (1953). Sample was homogenized in 4% TCA and harvested; supernatants were treated with a pinch of activated charcoal and incubated at 37 ºC for 15 minutes. Reaction mixture was centrifuged at 6000 rpm for 10 min to remove the charcoal residue. Supernatant was used to estimate vitamin C.

The estimation of vitamin E (α-tocopherol) was performed using the method described by Rosenberg (1992). Sample was mixed slowly with 0.1 N sulphuric acid and incubated at room temperature for overnight. The reaction mixture was filtered through Whatman No. 1 filter paper and filtrate was used for the estimation of vitamin E.

2.7 Statistical analysis

All the experiments were performed in triplicates. The data collected were analyzed by using GraphPad Prism software.

3 Results

The fruit bodies obtained from different substrates were harvested at the end of experiment. The maximum number of fruit bodies were found in case of substrate wheat straw, followed by 50% wheat straw + 50% paddy straw. The fruit bodies produced on wheat straw substrate had maximum diameter (8.55 cm ± 0.186 cm) as

compare to other substrate. Small and tiny fruit bodies were found in case of lawn grass as substrate (Figure 1). The yield of mushroom was measured from per kg of substrate used. Fresh weight and dry weight of mushroom produced on all the substrates were weighed and the maximum amount of fresh (29.27 g/kg substrate ± 0.75 g/kg) as well as dry weight (7.32 g/kg substrate ± 0.161 g/kg) was weighed those grown in wheat straw as substrate (Figure 2).

Vitamins A, C and E were estimated from fresh fruit bodies and powdered samples of *P. ostreatus* grown in wheat straw as substrate, as maximum yield was found in this substrate. Maximum amount of vitamins were found in case of fresh fruit bodies as compare to powdered sample or dry fruit bodies (Figure 3). Vitamin E (5.93 mg/g dry fruit body ± 0.098 mg/g; 7.23 mg/g fresh fruit body ± 0.111 mg/g) was estimated to be more than vitamin A (0.282 mg/g dry fruit body ± 0.004 mg/g; 0.363 mg/g fresh fruit body ± 0.004 mg/g) and vitamin C (0.277 mg/g dry fruit body ± 0.0015 mg/g; 0.363 mg/g fresh fruit body ± 0.0025 mg/g) in both samples.

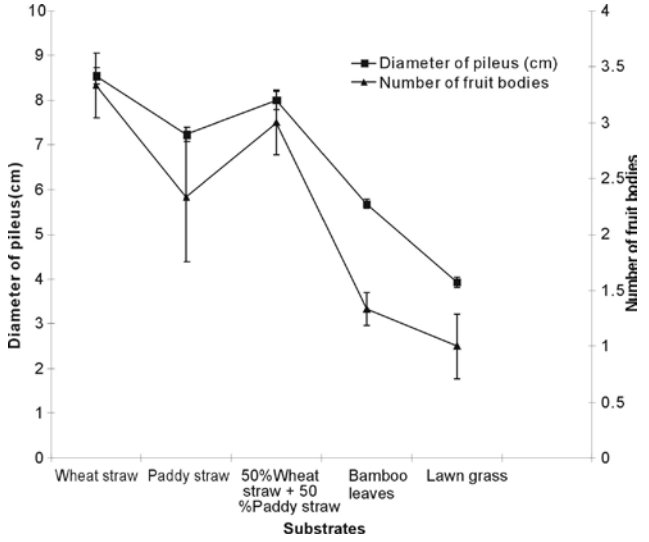

Figure 1. Number of fruit bodies and diameter of pileus of *P. ostreatus* grown in different substrates.

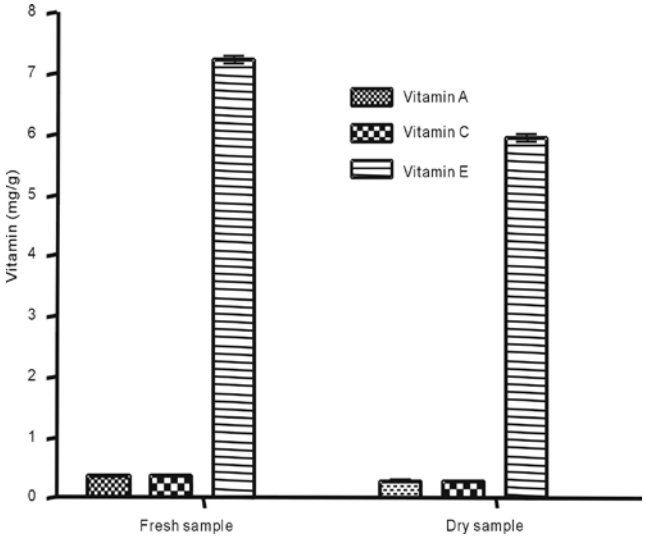

Figure 3. Amount of vitamins (A, C and E) present in fresh and dry powdered sample of *P. ostreatus*.

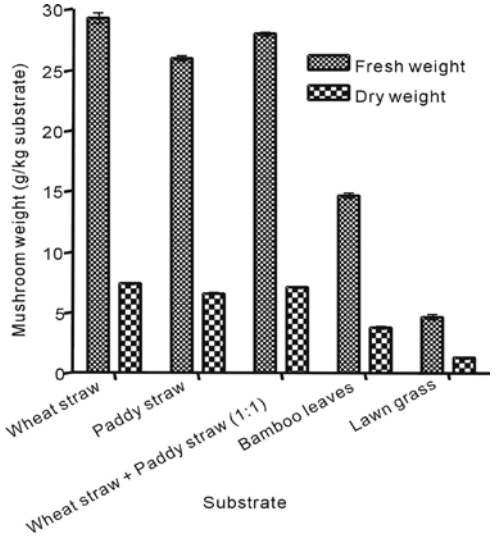

Figure 2. The fresh weight and dry weight of *P. ostreatus* grown in different substrates.

4 Discussion

Five different types of substrates were compared with respect to production of *Oyster mushroom* (*P. ostreatus*). Out of all of these substrates used, wheat straw was found to best suitable for the production of mushroom. Number of fruit bodies and diameter of pileus were also found better in this substrate. The fruiting bodies appeared 3 – 6 weeks after pinheads formation and took around one month later after inoculation of spawn. These results are similar to findings reported by Quimio (1976, 1978) who said that fruiting bodies 3 – 4 weeks after inoculation of spawn. A maximum yield value in wheat straw confirms that it can be easily used in the production of mushroom. One of the advantage of using this substrate is its availability and economic. Lawn grass was not found to good substrate for the production, as maximum number of bacterial as well as fungal contamination was present in this substrate. One of the reasons

might be improper sterilization of grass and presence of more green grass rather than dry one.

Mushrooms are high in protein, vitamins and essential elements including calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, manganese and selenium. Mushrooms are a group of fungi with good source of high quality proteins, rich in vitamins and minerals and high quality proteins, rich in vitamins and minerals and low calorie and cholesterol free (Selvi *et al*, 2006). From the present study it can be said that both fresh and dry fruit bodies possess non-enzymatic antioxidant activities, but the maximum activities were found in case of fresh fruit bodies. So in order to get maximum of these activities, these fruit bodies should be stored in cold condition. Vitamins have tremendous role from medicinal point of view and due to these reasons mushroom can be consumed as a source of vitamins. Vitamin A is necessary for clear vision in dim light. It also maintains the integrity of epithelial tissue (Gopalan *et al*, 2000). Vitamin C acts as the first line natural antioxidant and also acts as a free radical scavenger (Maxwell, 1995; Sies, 1993). Vitamin E prevents the attack of reactive oxygen species of the membrane PUFA (Sies, 1993). It also enhances immune response and resistance to diseases (Bendich, 1997).

Overall, wheat straw can be used to get good yield to *Oyster mushroom*. *Oyster mushroom* can be consumed as one of the source of non-enzymatic antioxidant vitamins.

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