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RESEARCH ARTICLE

NUTRITIONAL VALUE AND BIOMASS YIELD OF THE EDIBLE MUSHROOM *PLEUROTUS OSTREATUS* CULTIVATED ON DIFFERENT WASTES IN EGYPT

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Abstract

Mushroom yield (number of fruit bodies, fresh biomass gain and pileus diameter) and biochemical composition (proteins, fats, carbohydrates, ash, fiber, moisture, metals and amino acids). *Pleurotus ostreatus* was evaluated using different natural wastes for cultivation either singly or in combination. The most suitable substrate for fresh, high number, large sized pileus yield with high nutritional facts was the combined mixture of wheat and rice straws (1:1). This mushroom was rich in proteins, fibers, carbohydrates, metals with low fat content. *P. ostreatus* cultivated in the combined mixture was found to be rich in amino acids with glutamic and aspartic acids being of the highest quantities. Paddy and bamboo leaves not suited the quality and quantity of mushroom gain.

Keywords: *natural wastes, P. ostreatus*

Introduction

Edible mushrooms such as *Pleurotus ostreatus* is popular and widely cultivated throughout the world mostly in Asia and Europe owing to its excellent flavour, taste and higher biological efficiency (Shah *et al*., 2004, Mane *et al*., 2007) it belongs to class *Basidiomycetes*, subclass *Hollobasidiomycetidae*, order *Agaricales* (Ibekwe *et al*., 2008). *Pleurotus* species are efficient lignin degraders which can grow on wide variety of agricultural wastes (Jandaik and Goyal, 1995). Oyster mushrooms are rich source of proteins, minerals (Ca, P, Fe, K and Na) and vitamin C, B-complex (thiamine, riboflavin, folic acid and niacin) (Çağlarırmak, 2007). Besides using oyster mushrooms as food, it can also be used industrially for mycoremediation purposes (Kumari and Achal, 2008). 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, Shah *et al*., 2004).

In the present study the efficiency of different natural substrates on the biomass production of the local edible mushroom *P. ostreatus* have been evaluated. The nutritional value of the resulted mushrooms will be determined.

Materials and Methods

Fungal culture, growth conditions and spawns preparation

P. ostreatus was obtained from Alexandria City for Science and Technology, Egypt. It was maintained on Potato Dextrose Agar (PDA) plate at temperature of 4°C and subcultured every four weeks. Fully grown culture of *P. ostreatus* was used for spawn production in a wide mouth bottle by solid state fermentation (SSF) 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 the wide mouthed bottle, plugged tightly and sterilized for two consecutive days at

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15 psi, 121°C for 90 min then inoculated with *P. ostreatus* culture. Bottle was incubated at temperature of 25°C for 15 days under dark condition to give an optimum time for spawn production.

Growth substrates

Five different substrates were used: rice straw, wheat straw, paddy straw, mixture of rice and wheat straw and bamboo leaves. They were selected because they are readily available, as agricultural wastes and cause environmental problem by their burning in the fields.

Preparation of the substrates for cultivation

All the five substrates were chopped and soaked in water overnight and drained, to contain a moisture content of approximately 70%. Urea and gypsum were mixed at the rate of 2% and 3%, respectively (on dry weight basis). Ingredients were then mixed and filled in sterilized plastic bags. Sterilized substrate bags were inoculated with grain spawn at a rate of 6% (w/w), and mixed layer wise then heat sealed, and transferred to the incubation room for spawn run. Pinholes were made in the bags manually for gases elimination. The bags were incubated at temperature of 20ºC 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.

Fruiting

After incubation of 20 days, bags were transported to the fruiting site. Colonization of mycelia was seen clearly by naked eyes in these bags. Bags were cutoff and hanged the whole colonized mycelia with substrate using thread to initiate primordial formation. 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 temperature of 20ºC. All the treatments with substrates were performed in triplicates.

Yield and fruit body analysis

The yield of the *P. ostreatus* on the different substrates was quantified by the number and size of the produced fruit bodies. The fruit bodies were harvested at the end of the experiment and number of fruit bodies, diameter, fresh and dry biomass of fruit bodies were evaluated by using conventional techniques.

Proximate analysis

Analysis of moisture, protein, fat, crude fiber, total carbohydrates, ash of samples were done by standard methods (AOAC, 1995).

Mineral Estimation

Calcium (Ca) in ashed samples was determined by atomic absorption spectrophotometry after mineralization by hydrochloric acid (M.F.A, 1982). Iron (Fe) in ashed samples was estimated using a 1, 10-phenanthroline spectrophotometric method (M.F.A, 1982). Sodium (Na) and Potassium (K) were extracted from dried samples by acids before being determined with an atomic absorption spectrophotometer (M.F.A, 1982). Phosphorus (P) was determined spectrophotometrically after treating the ashed sample solution with ammonium molybdate, metavanadate and nitric acid (M.F.A, 1982, Gujral *et al*., 1987).

Amino acid Analysis

The amino acid composition of each sample was determined using a high performance liquid chromatography (HPLC)-based amino acid analyzer (Agilent 1120 Compact LC, Egypt) with single binary pump; Variable Wavelength Detector and the column used was ZORBAX Eclipse C18 with a detection wavelength 450 nm with the Ezchrom elite compact compliance software for data processing. The standards for different amino acids were procured from Sigma, USA and calibration chromatogram was established for 22 known amino acids. Thus, a 0.05 mmol standard solution of each of the standard amino acids was prepared by dissolving the corresponding acid in distilled water and then a mixture was constituted by mixing 1ml of each of the 22 standard amino acid solutions and this was later used to establish the standard chromatogram. The mobile phase consisted of a 10 mM aqueous sodium phosphate (pH 6.8) solution (buffer solution A) mixed with acetonitrile, running in a gradient, starting with a mixture consisting of 5% acetonitrile in the buffer

solution, and ending with acetonitrile alone. The free amino acids in the standards and in the mushroom species were automatically derivatized by reacting with o-phthaldialdehyde under basic conditions to produce o-phthaldialdehyde derivatives in the reaction columns of the amino acid analyzer. Two derivatization reagent solutions were prepared as follows: to 10 ml of 0.01 M sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) buffer solution B (pH 9.1) were added 10 ml of *b* mercaptopropionic acid to make reagent solution I. Reagent solution II was prepared by mixing 10 ml of 0.01 M sodium borate ($Na_2B4O_7 \cdot 10H_2O$) buffer solution B (pH 9.1) with 10 mg of o-phthaldialdehyde (OPA) dissolved in 3 ml of ethanol. Solutions I and II were filtered through 0.45 mm membrane filters before use. Following derivatization, the buffer solution A (mixed in acetonitrile in a 2:1 v/v ratio), containing the derivatized amino acids, was transferred into the HPLC for separation at a temperature of 45°C, with 10 ml injection volume and a flow volume of 1.0 ml/min.

Statistical analysis

The Statistical Program for Scientific Studies package (SPSS 12.0 for windows; SPSS Inc.) was used to perform statistical analyses. Data are presented as means with standard deviations (mean± S.D). A *p* value of less than 0.05 was seemed to be statistically significant.

Results and Discussion

The maximum numbers of fruit bodies with large diameter (9 cm) of pileus were found in case of combined substrates of rice and wheat straws (1:1) followed by rice straw, whereas the least value obtained on bamboo leaves (Fig. 1).

Fresh and dry biomasses of mushroom produced on all the substrates were obtained and the maximum amount of fresh biomass (28.4 $g \cdot kg^{-1}$ substrate) and dry weight (7.7 $g \cdot kg^{-1}$ substrate) were gained from combined mixture of rice and wheat straws (Fig. 2). These results are in accordance with Quimio (1976, 1978) who found that wheat and rice straws are the most suitable substrate mixture for growing mushroom and (Kumari and Achal, 2008) who reported that maximum yield of oyster was obtained on wheat straw followed by paddy straw.

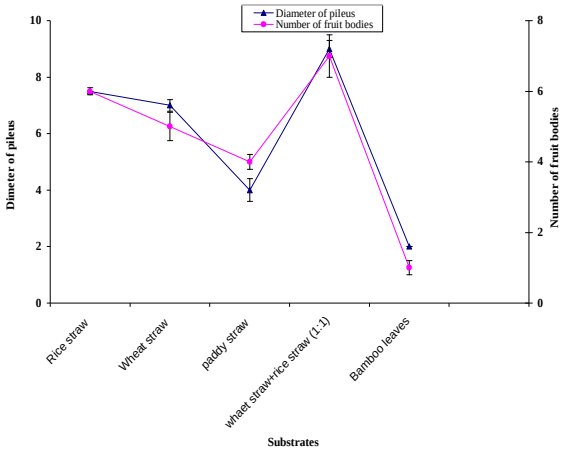

Figure 1. *Number of fruit bodies and pileus diameter of local P. ostreatus grown in different natural substrates straws*

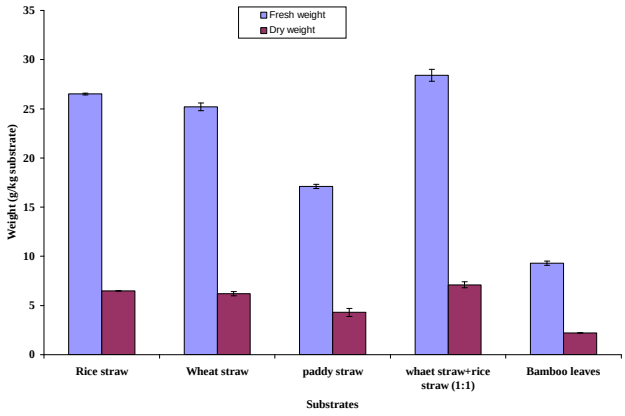

Figure 2. *The fresh and dry biomasses of local P. ostreatus grown in different natural substrates*

The data presented in Table 1 revealed that the nutritional value of the cultivated mushroom was found vary significantly with the substrate variation. The most favorable cultivation substrate was the combined mixture of rice and wheat straws (1:1). This mixture achieved the highest percentage of fat (3.9 %), protein (39.1 %), fiber (6.9 %) and calcium (350 mg %). Rice straw resulted in oyster with the highest moisture (90.1 %), carbohydrate (58.7 %), Fe (17.1 mg %) and K/Na ratio (7.2). The highest sodium content was obtained in *P. ostreatus* grown in wheat straw (305 mg %). The worst natural substrates for the nutritional value in oyster mushroom were paddy and bamboo straw

which may be due to its stiffness and difficult degradation by *P. ostreatus*. In that field, the use of different substrates for mushroom cultivation leads to different yield composition due to the biological and chemical difference between substrates (Laborde *et al*., 1993, Ragunathan and Swaminathan, 2003). Not only the protein content of the substrate but also the nature of protein in the substrate influences the protein content of the fruiting bodies. High protein of 23.9 g was determined in *P. ostreatus* and 24.6 g in *P. sajor-cajo* (Wang *et al*., 2001). Mushroom protein is an easily digested form. On a dry weight basis mushroom protein normally ranges between 20% and 40% which is better than many legumes such as soybeans and peanuts and protein-yielding vegetables foods (Chang and Buswell, 1996, Chang and Mshigeni, 2001). The differences in protein content depend on the C/N ratio in the cultivation substrate.

Table 1. *Characterization of P. ostreatus grown on different natural substrates*

Substrate	Moisture (%)	Fat (%)	Protein (%)	Fiber (%)	Carbo-hydrate (%)	Ash (%)	Ca mg%	Fe mg%	Na mg%	K mg%	K/Na Ratio
Rice straw	90.1	3.4	34.3	6.3	58.7	6.0	240	17.1	290	2100	7.2
Wheat straw	87.8	3.2	33.5	6.4	54.8	6.3	330	16.2	305	2000	6.8
Rice +Wheat straws (1:1)	88.7	3.9	39.1	6.9	56.7	6.5	350	16.9	295	2100	6.9
Paddy straw	88.5	3.1	32.0	6.2	50.9	6.1	260	15.8	270	1950	6.5
Bamboo leaves	87.4	3.3	31.9	6.1	46.6	6.3	221	13.2	280	1900	6.8
S.E ±	---	--	---	---	----	--	5.16	0.21	0.43	---	--

S.E – standard error

In this study high crude fibers (6.9 %) was obtained when *P. ostreatus* was grown on mixture of rice and wheat straw, while lower value (6.1 %) was resulted on bamboo leaves. *P. ostreatus* was rich in minerals which varied with different substrates either singly or in combination. Calcium concentration ranged from 221-350 mg %, Sodium from 270-305 mg %, Iron from 13.2-17.1 mg % and Potassium from 1900-2100 mg %. Minerals in diet are essential for metabolic reactions, healthy bones, transmission of nerve impulse, regulation of water and salt balance (Kalac and Svoboda, 2000). The calcium content of *P. ostreatus kumm* ranged from 240-330 mg % when grown was done on different natural substrates. Sodium and Potassium content differed moderately in *P. ostreatus* grown on different substrates. Iron variation with cultivation substrate was also reported by Kikuchi, 1984, Çağlarırmak, 2007, Syed *et al*., 2009.

Amino acid concentration of P. ostreatus

Table 2 shows the amino acid profile (mg/100g) of *P. ostreatus* cultivated on different natural substrates. A number of 18 types of amino acids were estimated in *P. ostreatus* but with different levels. The glutamic acid ranged from (51.2-63.3 mg/100 g). Aspartic acid from (28.6-41.3 mg/100 g) while Cystine from (3.3-6.1 mg/100 g) and Metheonin from (2-5.1 mg/100g) which is the least value. The highest quantity of amino acids was in case of glutamic acid, aspartic acid, leucine and threonine.

Purkayastha and Nayak (1981) reported that glutamic acid is one of the most abundant amino acids followed by leucine and arginine. *Pleurotus* species are rich in glutamic acid, aspartic acid, lysine, leucine and threonine (Mdachi *et al*., 2004). Under normal dietary conditions the amino acid profile of *P. ostreatus* studied confirms the high biological value of *P. ostreatus* protein.

Table 2. *Amino acid profile of local P. ostreatus grown on different natural substrates*

Amino acids (mg/100 g)	Rice straw	Wheat straw	Rice + wheat straws (1:1)	Paddy straw	Bamboo leaves
Alanine	25.2±0.14	27.3±0.09	28.4±0.11	20±2.1	15.1±0.05
Arginine	20.3±0.20	23.1±0.29	20.6±0.19	22±0.30	20.1±0.01
Aspartic acid	41.3±0.28	33.2±0.8	39.4±1.0	28.6±1.2	30±0.09
Cystine	4.3±0.5	3.3±0.1	3.9±0.7	6.1±0.6	5.2±0.5
Glutamic acid	63.3±1.2	58±0.9	69.5±2.1	59±1.8	51.2±1.1
Glycine	9.3±0.04	10.4±0.08	11.4±0.05	11.3±0.10	7.8±0.08
Histidine	10.9±0.3	12.2±0.6	11.6±0.1	19.4±0.3	13.1±0.6
Lysine	9.01±0.1	11.3±0.06	9.6±0.03	10.5±0.21	9.8±0.2
Methonine	2.9±0.1	2.6±0.3	2.08±0.02	5.1±0.02	3.2±0.6
Phenylalanin	13.6±0.19	18.3±0.2	20.1±0.3	17.4±0.3	16.3±0.1
Proline	7.4±0.17	8.1±0.04	9.2±0.1	10.3±0.21	15.8±0.2
Serine	12.2±0.3	14.1±0.3	17.4±0.1	18.5±0.2	12.6±1.1
Threonine	31.4±1.2	28.1±1.5	29.7±0.8	24.6±0.8	28.3±1.2
Tryptophane	9.1±0.2	8.9±0.09	6.9±0.3	5.2±0.04	4.7±0.3
Tyrosine	11.3±0.4	8.9±0.32	12.4±0.3	10.6±0.6	9.8±0.1
Valine	27.5±0.8	28.3±2.1	32.3±1.1	30.1±1.2	24.6±0.8
Leucine	29.9±0.9	24.4±0.5	37.1±1.2	20.4±0.1	19.8±0.5
Isoleucine	9.1±0.05	9.8±0.04	12.4±0.3	10.4±0.3	9.5±0.08

Conclusions

The combination of rice and wheat straws can be used to gain good production of nutritionally rich edible mushroom *P. ostreatus* in Egypt. In addition it is a useful mean of getting rid of the agricultural wastes. Mushroom can be considered as an alternative source of protein as it contain large quantities of essential amino acids and this can solve problems of vegetarian people all over the world who often suffer from protein deficiency.

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