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Original Article

Comparative Study on Nutritional Composition of Oyster Mushroom (*Pleurotus ostreatus* Fr.) Cultivated on Different Sawdust Substrates

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ABSTRACT: Effects of various sawdust substrates, namely Fig tree (T_2), Rain Tree (T_3), Mahogany tree (T_4), Ipil ipil tree (T_5), Eucalyptus tree (T_6) and mixture of all sawdust (T_1), supplemented with 30% wheat bran and 1% lime were analyzed on the growth and nutritional composition of *Pleurotus ostreatus* mushroom. The highest amount of carbohydrate (42.36%), calcium (31.98 mg/100g) and magnesium (19.85 mg/100g) were found in the T_4 sawdust substrate treated mushrooms. Whereas, moisture (90.20%), ash (13%), phosphorous (0.91%) and molybdenum (14.76 mg/100g) were highest for the T_1 substrate treated mushrooms. The highest amount of dry matter (10.53%), lipid (4.46%), nitrogen (4.52%), iron (42.55 mg/100g), zinc (27.65 mg/100g) and selenium (6.77 mg/100g) were obtained for T_2 substrate treatment. The highest amount of crude fiber (20.53%) and the lowest lipid (3.43%) was found for T_6 substrate. Protein (27.30%) and potassium (1.28%) were found to be highest for T_5 substrate treatment. However, the highest level of cobalt (22.40 mg/100g) was found for T_3 substrate treatment. Among all aspects, best nutritional composition containing mushroom was grown on T_2 sawdust substrate, followed by T_1, T_5, T_6, T_3 and T_4.

KEYWORDS: *Pleurotus ostreatus*, edible mushrooms, nutrition, proximate composition, saw dust, wheat bran.

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INTRODUCTION

Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease.[1] Edible mushrooms such as *Pleurotus ostreatus* is popular and widely cultivated throughout the world mostly in Asia and Europe owing to its excellent flavor, taste and higher biological efficiency.[2,3] Oyster Mushroom is widely cultivated in Bangladesh because the weather and climate of Bangladesh is suitable for its cultivation. As a vegetable, mushroom can play an important role to meet up the nutritional requirements of the population of our country.

Oyster Mushrooms are valuable health food, which are low in calories, high in vegetable proteins, zinc, chitin, fiber and vitamins (C, D and B-complex).[4] Oyster Mushroom contains 19-35% protein on dry weight as compared to 7.3% in rice 13.2% in wheat and 25.2% in milk.[5] It contains 4.0% fat having good quantity of unsaturated fatty acids which are essential in our diet.[6] Mushrooms are a valuable source of dietary fiber; 100g serving of mushrooms contains 2.5g dietary fiber. It is also rich in essential minerals and trace elements.[7] Mushrooms are one of the richest natural sources of selenium, which is vital for human health. Selenium is involved directly in the protection of cell walls from oxidation by free radicals. Selenium also enables thyroid to produce thyroid hormone and helps in lowering the risk of joint inflammation. Oyster Mushroom contains cobalt which is required in the synthesis of vitamin B_{12}.

Several species of mushrooms are of great importance because of their medicinal importance, for example, they are active against hypercholesterolemic conditions, hypertension, diabetes, cancer, infections etc.[8-15] Substrate plays an important role in the yield and nutrient contents of Oyster Mushroom. The substrate on which mushroom spawn is grown affects the mushroom production.[16] Oyster Mushroom are reported to be easily grown on different lignocellulose wastes such as banana leaves, cereal straw, paper wastes, sawdust, rice, wheat straw and other agro-wastes. Remarkable variations have been observed in the nutritional contents of Oyster Mushroom grown on these different substrates.[2,17,18] Bhuyan in his

study observed that the proximate composition of Oyster Mushroom is greatly changed due to different supplement used in sawdust based substrates.[19] A huge amount of sawdust is produced in Bangladesh annually. We can easily use our waste sawdust substrate and produce notable amount of edible mushroom.

Thus, the objective of this work was to evaluate influence of locally available sawdust of different trees with wheat bran 30% and 1% lime on nutritional composition of Oyster Mushroom so as to find out the most suitable sawdust substrate for cultivation of *Pleurotus ostreatus.*

MATERIALS AND METHODS

The experiment was carried out at the Mushroom Culture House(MCH), Biochemistry laboratory of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh and National Mushroom Development and Extension Center (NAMDEC) laboratory, Savar, Dhaka, Bangladesh during July 2012 to December 2012. Fruiting body of Oyster Mushroom (*P. ostreatus* Fr.) was collected from NAMDEC. Oyster Mushroom was grown on sawdust of different trees supplemented with 30% wheat bran and 1% lime on the spawn packet.[19-21] All the chemicals used were collected from Merck (Germany), Wako Pure Chemicals Industries Ltd. and JHD (China). These chemicals were analytical, spectroscopic grade and were used without further purification unless otherwise specified. The sample was weighted by electric balance (*KEY: JY-2003; China*) *and* heated in a muffle furnace (*Nebertherm: Mod-L9/11/c6;* Germany). The amount of minerals was determined by atomic absorption spectrophotometer (*analytikjenanovAA 400P*; Germany), flame photometry (*PFP7*; Germany) and spectrophotometer (*HALO BD-20S*; Germany).

Cultivation and harvesting of Pleurotus Ostreatus

Pleurotus ostreatus mashroom was grown on different sawdust substrates, namely, *Ficus carica* (Fig tree, T_2), *Albizia saman* (Rain Tree, T_3), *Swietenia mahagoni* (Mahogany tree, T_4), *Leucaena leucocephala* (Ipil ipil tree, T_5), *Eucalyptus globules* (Eucalyptus tree, T_6) and the mixture of all the sawdust (T_1), supplemented with 30% wheat bran and 1% lime in the spawn packets.[20-22] Study on the nutritional composition of the mushroom was done after harvesting the mushroom samples.

Proximate analysis of the mushrooms

Mushrooms grown from the spawn were collected packet wise and all the wastes and dusts were removed from the fruiting body. The proximate analysis of the mushroom of total experiment was conducted with the determination of moisture, dry matter, crude fiber, total fat, total carbohydrate, total ash, protein and determination of mineral content.

Determination of moisture and dry matter

Moisture amount was determined by keeping weighed quantity of sample in a thermostat controlled oven at 105ºC for 6 hours.[23-24] The dry weight of each sample was taken on an electric balance. The percentage of the moisture content and dry mater was then calculated by the following formula:

$$\text{Moisture (\%)} = \frac{\text{Initial Wt.} - \text{Final Wt.}}{\text{Original weight of sample}} \times 100$$

$$\text{Dry Matter (\%)} = 100 - \text{Moisture (\%)}$$

Total fat estimation

Crude fat was estimated by extracting the dry materials with diethyl ether solvent. The solvent was removed by using rotator evaporate.[24] The percentage of crude fat content was calculated by the following equation:

$$\text{Crude Fat (\%)} = \frac{\text{Weight of ether extract}}{\text{Weight of dried sample}} \times 100$$

Determination of crude fiber

10g of moisture and fat-free sample was heated at 80°C for about 0.5h with 200mL 0.25N sulphuric acid solution. The boiling medium volume was kept constant by frequent addition of hot water. The mixture was then filtered through a muslin cloth and the residue washed with hot water to get it free from acid. The residue was then treated with 200mL 0.32N NaOH solution and again washed with hot water, alcohol and ether to remove the alkali. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (W_e) on electric balance. The crucible was then heated in a muffle furnace at 600°C for 5-6 hours, cooled and weighed again (W_a). The difference in the weights ($W_e - W_a$) represents the weight of crude fiber.[24,25]

$$\text{Crude Fiber (\%)} = \frac{\text{Dry weight after digestion } (W_e) - \text{ Weight of ash } (W_a)}{\text{Weight of moisture and fat free sample}} \times 100$$

Determination of total ash

Ash content was determined by igniting previously dried sample in a muffle furnace at 600°C for 5-6 hours.[25] The ash content was calculated by the following equation:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of dried sample}} \times 100$$

Determination of protein

Total nitrogen was estimated by following the standard Kjeldahl method.[26]

$$\text{N (\%) in the supplied sample} = \frac{(V_a \times N_a - V_b \times N_b) \times 1.401}{W}$$

Where,

V_a = mL HCl measured in the conical task in the distill (usually 20.00 mL)
V_b = mL NaOH used for titration of the content in the conical flask
N_a = Normality of the HCl measured into the conical flask
N_b = Normality of the NaOH used for titration
W = g of mushroom powder used for the analysis

Crude protein content was obtained by multiplying the total nitrogen value by the conventional factor 6.25.[26] The percentage of protein in the sample was calculated by the following equation:

$$\text{Crude Protein (\%)} = \text{\% N} \times 6.25$$

Total carbohydrate estimation

The content of the available carbohydrate was determined by the following equation:[27]

$$\text{Crude Carbohydrate (\%)} = [100 - (\text{crude protein} + \text{crude fat} + \text{crude fiber} + \text{ash})]$$

Determination of approximate composition of mineral content of the mushrooms

The sample was digested with nitric acid to release of Ca, Mg, K, Fe, Zn, Co, Mo, Se and P. Ca, Mg, Fe, Co, Mo, Se and Zn were determined by atomic absorption spectrophotometer, K was determined by flame photometry and P was determined by spectrophotometer.[28,29]

Digestion

0.5g of dried sample was taken into each of 18 nitrogen digestion tubes and two tubes were kept blanks. 5 mL concentrated nitric acid were added to each of all 20 tubes. The tubes were placed in the digester and heated overnight at 125°C. At least after 4h of heating boiling was started and to complete digestion heating was continued. Every tube was observed to avoid drying. After cooling, the digestion mixture was transferred to 100 mL volumetric flask and distilled water was added up to the mark of volumetric flask. Filtration was performed on a dry filter paper into a dry bottle, which could be closed with a screw cap. Keeping the filtrate in the closed bottle, Ca, Mg, K, Fe, M Zn, Co, Mo, Se and P were determined in the filtrate.

Estimation of Ca and Mg

20 mL diluted filtrate was transferred into a 50 mL volumetric flask using a pipette. 5 mL $LaC1_3$ solution was added and distilled water was added up to the mark of volumetric flask and mixed. Then the content of Ca and Mg was measured by atomic absorption spectrometer.

$$\text{Ca or, Mg (mg/kg sample)} = \frac{a \text{ X } 25000}{b \text{ X } c}$$

Where,

a = mg/L Ca or Mg, measured on atomic absorption spectrometer
b = mL diluted filtrate transferred into the 50 mL volumetric flask for determination of Ca or Mg
c = g sample weighed into the digestion tube

Estimation of K and P

The content of K was measure by flame photometer. 10 mL diluted filtrate was transferred into a 50 mL volumetric flask, distilled water was add up to the mark and mixed. Then the absorbance was recorded by flame photometer.[29] The P content was measured by spectro-photometer. 10 mL diluted filtrate was transferred into a 50 mL volumetric flask. 30 ml water was added, mixed and then 10 mL ammonium molybdate-ascorbic acid solution was added to the flask and mixed. After 15 minutes, the absorbance was measured on a spectrophotometer at 890 nm.[29]

$$\text{K or, P (mg/kg sample)} = \frac{a \text{ X } 25000}{b \text{ X } c}$$

Where,

a = mg/L K or P measured on flame photometer or spectrophotometer
b = mL diluted filtrate transferred into the 50 mL volumetric flask for determination of K or P
c = g sample weighed into the digestion tube

Estimation of Fe, Zn, Co, Mo and Se

The contents of Fe, Mo, Zn, Co and Se elements were measured by atomic absorption spectrometer (AAS) directly in the undiluted filtrate.[29,30]

$$\text{Fe, Zn, Co, Mo, Se (mg/kg sample)} = \frac{d \text{ X } 100}{c}$$

Where,

d = mg/L micro nutrient (Fe, Zn, Co, Mo and Se) measured on atomic absorption spectrophotometer
c = g sample weighed into the digestion tube

Statistical data analysis

Data on various parameters were analyzed by standard statistical method using SPSS 12.0 statistical software and Microsoft Office Excel 2007. Computer package program MSTAT-c was also used. The experiment was laid out in single factor Randomized Complete Block Design. The experiment considered 6 treatments with 3 replications and 1 spawn packets in each replication. The analysis of variance was conducted and means were separated and compared by least significant difference (LSD) and DMRT test respectively. Significance level was considered as 5% for the tests.

RESULTS AND DISCUSSION

Effect of Different Sawdust Substrates on Proximate Analysis of Oyster Mushroom

***Effect on moisture and dry matter content*:** All the treatments were statistically similar but numerically different to each other as shown in Table 1. Numerically the moisture percent ranged from 90.20-89.47. The highest moisture percent moisture was observed in treatment T_1 (90.20) followed by T_6 (90.17), T_3 (90.13), T_5 (90.13) and T_4 (89.97) treatments and the lowest moisture was in T_2 (89.47) (Table 1). The moisture percentage of Oyster Mushrooms grown on different sawdust substrates observed in this study are supported by earlier studies by Moni *et al* (88.15-91.64%)[31] and Alam *et al* (87- 87.5%).[30] Numerically the dry matter percent of fruiting body ranged from 10.53-9.80. The highest dry matter percentage was observed in treatment T_2 (10.53) followed by T_4 (10.03). The other treatments were statistically similar while the lowest dry matter percentage was found for T_1 (9.80) (Table 1). Similar results were observed with previous studies by Ahmed *et al*[32] who found that the dry matter of the fruiting bodies ranged from 9.40 to 9.98 when Oyster Mushroom was grown on saw dust supplemented with cow dung.

***Effect on protein and lipid content*:** The content of protein varied from 27.30-25.35% (w/w) in the mushroom grown on different sawdust substrates. The highest content of protein was found in treatment T_5 (27.30%) and the lowest protein was found in T_2 (35.35%) as shown in Table 1. The result of the present study corroborates with the studies of Chang *et al*[33] who reported that the fruit bodies of Oyster Mushrooms contained 26.6-34.1% protein. The lowest lipid percentage was counted under treatment T_6 (3.43) and the highest lipid percentage was counted under T_2 (4.46) (Table 1). The lipid percentage results of this study varied significantly with previous results from Chang *et al* (1.1-8.0),[33] Moni *et al* (1.49-1.90)[31] and Alam *et al* (4.30-4.41).[30]

***Effect on carbohydrate and crude fiber content*:** The lowest percentage of carbohydrate was observed under treatment T_1 (39.67) and the highest carbohydrate percentage was observed under T_4 (42.36) (Table 1). The finding of this study does not maintain the Chang *et al*

study[33] who reported that the fruit bodies of mushrooms contained 40.3-50.7% of carbohydrates. But the results match the study of Alam *et al*[30] who found 39.82-42.83% of carbohydrates in *Pleurotus spp.* The highest percentage of crude fiber was observed under treatment T_6 (20.53) and the lowest crude fiber percentage was observed under T_1 (17.13) (Table 1). The findings of the present study corroborate with the study of Alam *et al*[30] who reported 8.7-23.29 g/100g of fiber in *Pleurotus spp.*

***Effect on ash content*:** The highest percentage of ash was observed in the treatment T_1 (13) followed by T_2 (11), while the lowest percentage of ash was in the treatment T_5 (8.5) (Table 1). The findings of the present study are supported by the study of Khlood-Ananbeh *et al*[34] who reported ash contents were moderate in the fruiting bodies. Alam *et al*[30] reported 8.28-9.02% of ash in *Pleurotus spp.* The higher amount of ash found in the present study may be due to the newly introduced varieties.

Different sawdust substrates had an almost similar effect on the approximate composition of Oyster Mushroom which is shown in Figure 1.

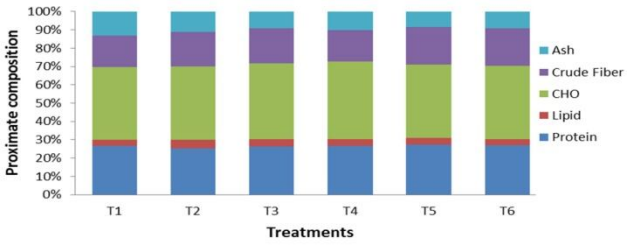

Figure 1. Effect of different sawdust substrates on proximate composition analysis of dry matter of Oyster Mushroom (*P. ostreatus*).

Table 1. Effect of sawdust substrates on proximate composition of Oyster Mushroom (*Pleurotus ostreatus*).

Treatments	Moisture (%)	Dry Matter (%)	Protein (%)	Lipid (%)	CHO (%)	Crud fiber (%)	Ash (%)
T_1	90.20a	9.80a	26.46ab	3.47cd	39.67c	17.37d	13.0a
T_2	89.47a	10.53a	25.35b	4.46a	40.19bc	18.96c	11.0a
T_3	90.13a	9.87a	26.24ab	4.25a	41.26b	19.25b	9.0bc
T_4	89.97a	10.03a	26.73ab	3.75b	42.36a	17.13d	10.0b
T_5	90.13a	9.87a	27.30 a	3.67bc	40.23bc	20.30b	8.5c
T_6	90.17a	9.83a	26.83ab	3.43d	40.21bc	20.53a	9.0bc
CV (%)	0.48%	4.37%	10.02%	3.39%	1.79%	1.15%	5.22%
$LSD_{(0.05)}$	0.80	0.80	4.91	0.23	1.32	0.39	1.02

Means followed by same letter significantly different at 1% or 5% level of significance.

Effect of Different Sawdust Substrates on Major Mineral Content of Oyster Mushroom

***Effect on nitrogen, phosphorus, potassium content*:** All the treatments were statistically and numerically different to each other as shown in Table 2. The highest percentage of nitrogen was observed under treatment T_2 (4.52) and the lowest nitrogen percentage was observed under T_3 (4.03). The findings of the present work was supported with the study of Moni *et al*[31] who analyzed for various nutritional parameters and found 4.22-5.59% of nitrogen on dry matter basis in fruiting bodies of Oyster Mushroom. The highest percentage of phosphorus was observed under treatment T_1 (0.91) and the lowest phosphorus percentage was observed under T_4 and T_3 (0.77) (Table 2). The finding of the present work differs with the study of Chang *et al*[33] that reported the 5.87-8.40 mg/g of phosphorus on dry weight of fruiting bodies. However, the result is supported by the study of Sarker *et al*[22] who found 0.97% phosphorus in Oyster Mushrooms grown on sawdust based substrates. The highest percentage of potassium was observed under treatment T_5 (1.28) and the lowest potassium percentage was observed under T_1 (1.13) (Table 2). The finding of the present work matches with the studies of Chang *et al* (1.432-1.88 mg/g)[33] and Sarker *et al* (1.3%)[22].

***Effect on calcium and magnesium content*:** The highest milligram percentage of calcium was observed under treatment T_4 (31.98) and the lowest milligram percentage calcium was observed under T_2 (27.33) (Table 2). This finding is similar to previous report by Alam *et al*[30] who found 22.15-33.7 mg/100g of calcium in different Oyster Mushroom varieties. Sarker *et al*[19,22] found 2400 ppm calcium in Oyster Mushroom grown on sawdust based substrates. The highest milligram percentage of magnesium was observed under treatment T_4 (19.85) and the lowest milligram percentage magnesium was observed under T_2 (13.31) (Table 2). Similar results were reported earlier Alam *et al*[30] who found magnesium in 13.4-20.22 mg/100g range.

Table 2. Effect of sawdust substrates on major mineral contents of Oyster Mushroom (*Pleurotus ostreatus*).

Treatments	N (%)	P (%)	K (%)	Ca (mg/100g)	Mg (mg/100g)
T_1	4.36ab	0.91a	1.13c	31.25ab	15.23c
T_2	4.52a	0.88a	1.18bc	27.33b	13.31d
T_3	4.03b	0.77b	1.26ab	30.69ab	18.02b
T_4	4.39ab	0.79b	1.27a	31.98a	19.85a
T_5	4.16ab	0.85ab	1.28a	31.92a	14.35cd
T_6	4.12b	0.87a	1.16c	31.47a	17.26b
CV (%)	4.98%	4.84%	3.83%	7.05%	4.36%
$LSD_{(0.05)}$	0.39	0.81	0.81	3.95	1.30

Means followed by same letter significantly different at 1% or 5% level of significance.

Effect of Different Sawdust Substrates on Micro Mineral Content of Oyster Mushroom

***Effect on* iron (mg%), zinc (mg%) and cobalt (mg%) *content*:** All the treatments were statistically and numerically different to each other as shown in Table 3.

The highest amount (mg%) of iron was observed under treatment T_2 (42.55) and the lowest iron content was observed under T_4 (37.87) (Table 3). The findings of the present study matches with Alam *et al*[30] who found 33.45-43.2 mg% of iron in different Oyster Mushroom varieties. Sarker *et al*[20] found 92.09-118.40 ppm iron in Oyster Mushroom grown on sawdust based substrates. The highest amount (mg%) of zinc was observed under treatment T_2 (27.65) and the lowest amount was observed under T_6 (20.56) (Table 3). The result of the present study matches with the study of Alam *et al*[30] who found 16-20.9 mg% of zinc in different Oyster Mushroom varieties. Sarker *et al*[22] found 30.92 ppm zinc in Oyster Mushroom grown on sawdust based substrates. The highest amount (mg%) of cobalt was observed under treatment T_2 (22.40) and the lowest amount was observed under T_5 (11.80) (Table 3).

***Effect on* molybdenum (mg%) and selenium (mg%) *content*:** The highest amount (mg%) of molybdenum was observed under treatment T_1 (14.76) and the lowest amount was observed under T_5 (5.82). The highest amount (mg%) of selenium was observed under treatment T_2 (6.77) and the lowest amount was observed under T_4 (4.67) (Table 3).

Table 3. Effect of sawdust substrates on micro mineral contents of Oyster Mushroom (*Pleurotus ostreatus*).

Treatments	Fe (mg/100g)	Zn (mg /100g)	Co (mg/100g)	Mo (mg/100g)	Se (mg/100g)
T_1	39.93b	25.90ab	12.80d	14.76a	4.67b
T_2	42.55a	27.65a	14.40cd	7.48cd	6.77a
T_3	40.25b	26.45ab	22.40a	8.83bc	5.77ab
T_4	37.87c	22.39bc	16.57bc	9.64b	4.67b
T_5	40.23b	20.73c	11.80d	5.82d	4.70b
T_6	41.8ab	20.56c	18.40b	7.07cd	5.60ab
CV (%)	2.45%	11.39%	9.47%	11.04%	17.88%
$LSD_{(0.05)}$	1.80	4.96	2.79	1.79	1.74

Means followed by same letter significantly different at 1% or 5% level of significance.

CONCLUSION

Effect of various sawdust substrates on the nutritional composition of *P. ostreatus* mushroom was analyzed. Among the all treatments the highest amount of carbohydrate, calcium and magnesium were observed in T_4 substrates. The highest amount of moisture, ash, phosphorous, and molybdenum were observed in control treatment mixture of all five tree sawdust mixture (T_1) substrate. The highest amounts of dry matter, lipid, nitrogen, iron, zinc, selenium were observed in T_2 substrate. The highest amount of crude fiber was observed in T_6 substrate. The highest amount of protein and potassium were observed in T_5 substrate. The highest amount of cobalt was observed in T_3 substrate. Therefore observing the nutritional composition it can be concluded that T_2 substrate is the best among the applied treatments for locally grown popular *Pleurotus ostreatus* mushroom variety in Bangladesh.

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