

Nutrient and Antinutrient Composition of *Pleurotus ostreatus* Grown on Different Substrates [†]

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Abstract: There is a global need for alternative sources of high-quality, protein-rich foods to combat rising food insecurity and malnutrition. This study compared the nutrient/anti-nutrient composition of Oyster mushrooms grown on different substrates (rice bran + saw dust (Ms/r) (1:2) or ground banana leaves (Mb)). Ms/r had significantly higher yield as well as carbohydrate Na, Cl and Phytate contents compared to Mb. On the other hand, protein, K, vitamins B1 and D and Oxalate contents of Mb were significantly higher than those of Ms/r. The result indicated that banana leaves may be a good substrate for nutritionally beneficial mushrooms.

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1. Introduction

There is a global threat to food production and security as a result of the adverse effects of a constantly changing climate with Africa one of the worst hit due to increasing population, and reduced agricultural yields resulting from overstretched lands [1,2]. Also the negative impact of the COVID-19 pandemic on meat supply chain further highlights the urgent need for alternative sources of high-quality, protein-rich foods to combat rising food insecurity and malnutrition. To combat the rising issues in food security and nutrition on the African continent, an efficient, economic, and technology-based approach is required [2]. Recent advances in biotechnological processes take advantage of the adaptive characteristics of microorganisms to breakdown lignocellulosic agro-industrial wastes that would otherwise have constituted environmental nuisances. Oyster mushrooms (*Pleurotus ostreatus*) cultivation is a viable agricultural practice that presents with the advantage of less growth requirement including growing on agricultural wastes, requiring less space and water compared to other crops as well as their short growth time. Oyster mushrooms are highly nutritious, with low calorie and high protein content comparable to egg, milk, and meat [3]. They are also a rich store of various minerals and vitamins making them a treasure to dieticians [4]. It has been reported that the nutritional composition of mushrooms depends largely on the type and nutrient composition of the substrate [2,5]. This is as a result of the variation in nutritional value of oyster mushrooms when cultivated on different substrates such as cotton seed hulls, perilla stalks, rice and

wheat bran, etc. [6–8]. This study therefore, investigated the nutrient and anti-nutrient composition of mushrooms grown on different substrates.

2. Materials and Methods

Chemicals and reagents: All chemicals and reagents used in this study were products of BDH (India), May and Bakers (England), Sigma Aldrich (USA), Merck (Germany) and were of analytical grade.

Plant materials: The plant materials used in this study include *P. ostreatus* and banana leaves, mahogany saw dust and rice bran.

Collection and processing of substrate materials: Wood sawdust, rice bran, and banana leaves were collected in Nsukka, dried and milled to fine particles.

Spawn preparation: The mushroom spawns were inoculated on parboiled sorghum seeds and were maintained as stock culture for mushroom cultivation [9].

2.1. Substrate Preparation and Mushroom Cultivation

In order to prepare 42 kg of substrate (saw dust and rice bran (2:1) or banana leaves), 32 litres of water containing 0.42 kg of CaCO₃ were utilized. The substrate mixture was bagged in transparent heat-resistant polythene bags and tied before sterilization at 121 °C and 15 psi for 15 min. The sterile bagged substrates were allowed to cool to room temperature before the stock culture (spawn) was aseptically inoculated into the bagged substrates. The inoculated bags were tied up and allowed some days (15–21 days) to ramify in the dark. Total colonization of the inoculated substrate bags with fungi hyphae and the appearance of pin-heads on the bags were visible evidence of complete ramification. Incisions were gently made aseptically on the ramified mushroom bags before they were transferred to the growth room for production of fruiting body [10]. The relative humidity maintained in the cultivation room during the experiment was 75–85% under room temperature. The humid air in the dark room was achieved by sprinkling water to the air, wetting the floor and keeping water in trays. The matured fruiting bodies were harvested and used for analysis.

2.2. Percentage Yield/Biological Efficiency

Harvested mature mushrooms were weighed with analytical balance to determine the biological efficiency (BE) of mushrooms produced from substrates. The average BE of harvests was calculated using formula below, and comparison was made among different substrates [11]

$$BE = \frac{(\text{weight of fresh mushroom fruiting body})}{(\text{weight of dry substrate})} \times 100$$

2.3. Determination of Proximate Composition

Proximate composition of Ms/r and Mb were analysed using the method of AOAC [12] while protein determination was carried out using the micro-Kjedhal's method as described by Pearson [13]

2.4. Determination of Minerals and Vitamins Composition of the Mushrooms

The mineral and vitamins content of the mushrooms were determined according to methods of AOAC [14] and AOAC [12] respectively.

2.5. Determination of Anti-Nutrients

Oxalate, phytate, and tannin contents were determined by the method reported by Munro [15], Lolas & Markakis [16], and Maxson & Rooney [17] respectively while haemagglutinin content was determined using the method described by AOAC [14].

2.6. Statistical Analysis

All primary data were analyzed using student T-test in IBM Statistical Product and Service Solutions (SPSS), version 23. The results were presented as mean \pm standard deviation and differences between means were assessed by Duncan's multiple range test (DMRT) and were considered statistically significant when $p < 0.05$.

3. Results

Table 1. Yield and biological efficiency.

Mushroom Sample	Yield (g)	Biological Efficiency (%)
Ms/r	1250.00	50
Mb	250.00	10

Table 2. Proximate composition of Ms/r and Mb.

Parameters (%)	Carbohydrate	Protein	Fat	Moisture	Fiber	Ash
Ms/r	14.16 \pm 0.23 ^b	2.75 \pm 0.29 ^a	2.96 \pm 0.06 ^a	72.22 \pm 0.09 ^a	4.93 \pm 0.08 ^a	3.00 \pm 0.04 ^b
Mb	8.01 \pm 0.02 ^a	8.43 \pm 0.23 ^b	2.87 \pm 0.02 ^a	73.98 \pm 0.19 ^a	4.61 \pm 0.08 ^a	2.12 \pm 0.16 ^a

Each value represents the means \pm standard deviations (n = 3); values within same column having same superscripts letters are not significantly different ($p < 0.05$).

Table 3. Mineral contents of Ms/r and Mb.

Mineral (mg/100g)	Ca	Fe	Na	K	Zn	Cl
Ms/r	47.00 \pm 9.52 ^a	1.67 \pm 0.26 ^a	79.35 \pm 1.63 ^b	459.42 \pm 1.37 ^a	0.29 \pm 0.043 ^a	121.86 \pm 1.64 ^b
Mb	53.04 \pm 2.35 ^a	1.61 \pm 0.04 ^a	70.15 \pm 1.63 ^a	574.48 \pm 0.74 ^b	0.32 \pm 0.00 ^a	108.28 \pm 2.51 ^a

Each value represents the means \pm standard deviations (n = 3); values within same column having same superscripts letters are not significantly different ($p < 0.05$).

Table 4. Vitamin content of Ms/r and Mb.

Vitamin (mg/100g)	Ms/r	Mb
Vit.B1	0.0750 \pm 0.0200 ^a	0.1480 \pm 0.0042 ^b
Vit. B2	0.1855 \pm 0.0007 ^a	0.2355 \pm 0.0404 ^a
Vit. B3	0.1450 \pm 0.0212 ^a	0.3900 \pm 0.1131 ^a
Vit D (IU)	104.07 \pm 22.96 ^a	134.83 \pm 25.22 ^b

Each value represents the means \pm standard deviations (n = 3); values within same row having same superscripts letters are not significantly different ($p < 0.05$).

Table 5. The anti-nutrient content of Ms/r and Mb.

Anti-nutrient(mg/100g)	Ms/r	Mb
Phytate	42.41 \pm 3.53 ^a	59.88 \pm 0.01 ^b
Oxalate	78.93 \pm 1.52 ^b	42.5 \pm 3.54 ^a
Tannins	198.04 \pm 3.16 ^a	203.32 \pm 4.43 ^a
Hemagglutinin	NIL	NIL

Each value represents the means \pm standard deviations (n = 3); values within same column having same superscripts letters are not significantly different ($p < 0.05$).

4. Discussion

Mature *Pleurotus ostreatus* fruiting bodies were harvested at 5.72 weeks and 7 weeks respectively, from Ms/r and Mb. Ms/r also gave higher mushroom yield compared to Mb as seen in Figure 1. The proximate analysis of the mushrooms showed comparable fats,

moisture, and fibre contents of both mushrooms while Ms/r had significantly higher carbohydrate content than Mb. However, the result indicates that Mb could be a better protein source due to its significantly higher protein content in comparison to Ms/r. Minerals and vitamins are food-derived micro-nutrients which are essential for many cellular and physiological processes such as enzyme catalysis, nerve conduction, blood pressure regulation, etc. The micro-nutrient analysis of the mushrooms showed that Ms/r had significantly higher Na and Cl contents compared to Mb. However, K, vit B1, and vit D contents of Mb were significantly higher when compared to Ms/r.



Figure 1. (a) Picture of *Pleurotus ostreatus* grown on saw dust and rice bran (1:2) (Ms/r) (b) Picture of *Pleurotus ostreatus* grown on banana leaves (Mb).

Anti-nutrients are chemical compounds synthesized in natural foods during metabolism, and their presence (depending on concentration) could inhibit the maximum utilization of nutrients especially proteins, vitamins and minerals present in food [18–20]. Phytate and oxalate are anti-nutrients that reduce the bioavailability of minerals such as calcium, forming insoluble salts of these minerals. The anti-nutrient analysis of the mushrooms revealed higher oxalate and significantly lower phytate contents in Ms/r when compared to those of Mb. The result indicated that banana leaves may be a good substrate for nutritionally beneficial mushrooms as indicated in the higher protein and vitamin contents of Mb.

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References

1. Frischen, J.; Meza, I.; Rupp, D.; Wietler, K.; Hagenlocher, M. Drought risk to agricultural systems in Zimbabwe: A spatial analysis of hazard, exposure, and vulnerability. *Sustainability* **2020**, *12*, 752.
2. Muswati, C.; Simango, K.; Tapfumaneyi, L.; Mutetwa, M.; Ngezimana, W. The Effects of Different Substrate Combinations on Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*). *Int. J. Agron.* **2021**, *2021*, 10.
3. Peter, O.E.; Peter, G.R.; Obele, I.I.; Owuna, G.; Danladi, M.M.; Obiekieze, S.; Akwashiki, O. Utilization of Some Agro-Wastes for Cultivation of *Pleurotus ostreatus* (Oyster Mushroom) in Keffi Nigeria. *Front. Environ. Microbiol.* **2019**, *5*, 60–69.
4. Atila, F.; Tuzel, Y.; Fernández, J.A.; Cano, A.F.; Sen, F. The effect of some agro– industrial wastes on yield, nutritional characteristics and antioxidant activities of *Herichium erinaceus* isolates. *Sci. Hortic.* **2018**, *238*, 246–254.
5. Hoa, H.T.; Wang, C.L.; Wang, C.H. The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology* **2015**, *43*, 423–434.
6. Wang, S.; Xu, F.; Li, Z.; Zhao, S.; Song, S.; Rong, C.; Geng, X.; Liu, Y. Scientia Horticulturae The spent mushroom substrates of *Hypsizigus marmoreus* can be an effective component for growing the oyster mushroom *Pleurotus ostreatus*. *Sci. Hortic.* **2015**, *186*, 217–222.
7. Kinge, T.R.; Adi, E.M.; Mih, A.M.; Ache, N.A.; Nji, T.M. Effect of substrate on the growth, nutritional and bioactive components of *Pleurotus ostreatus* and *Pleurotus florida*. *Afr. J. Biotechnol.* **2016**, *15*, 1476–1486.
8. Atila, F. Compositional changes in lignocellulosic content of some agro-wastes during the production cycle of shiitake mushroom. *Sci. Hortic.* **2019**, *245*, 263–268.
9. Thongklang, N.; Luangharn, T. Testing agricultural wastes for the production of *Pleurotus ostreatus*. *Mycosphere* **2016**, *7*, 766–772.
10. Chiejina, N.V.; Osibe, D.A. Oil palm fruit fibre promotes the yield and quality of *Lentinus squarrosulus* (Mont.) Singer, an edible Nigerian mushroom. *African J. Biotechnol.* **2015**, *14*, 1195–1200.
11. Tarko, D.B.; Sirna, A.M. Substrate optimization for cultivation of *Pleurotus ostreatus* on lignocellulosic wastes (coffee, sawdust, and sugarcane bagasse) in Mizan–Tepi University, Tepi Campus, Tepi Town. *J. Appl. Biol. Biotechnol.* **2018**, *6*, 14–20.
12. AOAC *Official Methods of Analysis of Association of Official Analytical Chemists*, 18th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 2010.
13. Pearson, D.; Cox, H.E. *The chemical analysis of foods*; 7th ed.; Churchill Livingstone: London, 1976; ISBN 0443014116.
14. AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17th ed.; AOAC: Washington, DC, USA, 2000.
15. Munro, A.B. Oxalate in Nigerian vegetables. *West Afr. J. Biol. Appl. Chem.* **2000**, *12*, 14–18.
16. Lolas, G.M.; Markakis, P. Phytic acid and other phosphorus compounds of beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **1975**, *23*, 13–15.
17. Maxson, E.D.; Rooney, L. Evaluation of methods for tannin analysis in sorghum grain. *Cereal Chem.* **1972**, *49*, 719–729.
18. Fekadu, H.; Fekadu, B.; Desse, G. Effect of Traditional Processing Methods on Nutritional Composition and Anti-nutritional Factors of Anchote (*Coccinia Abyssinica* (lam.) Cogn) Tubers Grown in Western Ethiopia. *J. Food Process. Technol.* **2013**, *4*, 1–8.
19. Gemed, H.F.; Ratta, N. Antinutritional Factors in Plant Foods: Potential Health Benefits and Adverse Effects. *Int. J. Nutr. Food Sci.* **2014**, *3*, 289.
20. Gemed, H.F.; Haki, G.D.; Beyene, F.; Woldegiorgis, A.Z.; Rakshit, S.K. Proximate, mineral, and antinutrient compositions of indigenous Okra (*Abelmoschus esculentus*) pod accessions: implications for mineral bioavailability. *Food Sci. Nutr.* **2016**, *4*, 223–233.