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Impact of Different Substrates on the Nutritional and Mineral Profile of *Pleurotus florida*: A Comparative Analysis

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Abstract

Edible mushrooms have been used as a source of food extensively for quite a long time. They are rich in protein, vitamins and are known to possess several health-related benefits. Edible mushrooms have been cultivated using various agro-industrial wastes. Being saprophytes, the nutrient composition of the mushrooms is extensively reliant on the composition of the substrate. In this perspective, the present study was undertaken to assess the role of different substrates in deciding the yield and nutritional content of the oyster mushroom, *Pleurotus florida*. *P. florida* was cultivated using different substrates such as rice straw, sugarcane bagasse, sawdust, groundnut shell and onion peel wastes using solid-state fermentation under controlled conditions. Further, the harvested mushrooms were subjected to morphological analysis and nutritional labeling such as crude protein, total carbohydrates, crude fiber, total lipids, and ash content using standard analytical methods. Mineral content such as calcium, magnesium, sodium, potassium, iron, zinc, manganese, copper, nickel, and selenium were estimated using Inductively Coupled Plasma – Mass Spectroscopic analysis. From the results, it can be concluded that the substrates used for mushroom cultivation not only affect the yield but also the nutritional and mineral profiles of the mushrooms. Hence, this strategy can be effectively adopted for the fortification of mushrooms thereby improving the bioavailability of required minerals for promotion of health and well-being.

Keywords: Oyster Mushroom, Mineral level, Nutritional Profile, Substrates, Food Fortification.

Introduction

Mushrooms are the fruiting bodies of macro fungi. In Tamil Nadu, three major types of mushrooms are being cultivated namely, milky mushroom, oyster mushroom and button mushroom (1). Among these mushrooms, oyster mushrooms can grow at a normal temperature ranging from 24-35°C (2). *Pleurotus florida* is a species of oyster mushroom belonging to the kingdom of fungi and phylum of Basidiomycota. It is an edible mushroom and is widely used as a source of food because of its higher nutrient content. Mushrooms are heterotrophic creatures that need external nutrients to thrive and the vegetative mycelium provides nutrients for growth (3). Being a saprophyte, the nutrient content of the mushroom solely depends on the substrate used for the cultivation process (4). Agricultural waste is one of the most available substrates used for mushroom cultivation. Substrates play a crucial role in the imparting the nutritional composition of oyster mushrooms

and variations have been perceived in proximate and mineral compositions of mushrooms cultivated using different substrates. Hence, in the present study, the duration of harvesting, the yield of first flush, proximate composition, and mineral content of *P. florida* cultivated using rice straw, sugarcane bagasse, groundnut shell, sawdust (large and small size) and onion peel as substrates were analyzed and compared.

Materials and Methods

Mushroom used

Pleurotus florida was chosen for the study. *P. florida* is a tropical and subtropical edible fungus. These mushrooms can use a wide range of agricultural wastes and convert lignocellulosic biomass into nutritious food. Mushroom spawns were procured from Dr. Mohan's mushroom farms, Madurai, Tamil Nadu, India.

Substrates used

Rice straw, sugarcane bagasse, groundnut shell, sawdust (small and large particle size), and onion peel waste were used for the cultivation of *P. florida.* Rice straw and groundnut shells were collected from a local farm near Dindigul, India. Sugarcane bagasse was procured from a local juice shop near Madurai, India and sawdust was collected from the carpentry of the institution campus. Both small and large-sized sawdust particles were used for mushroom cultivation to study the effect of particle size on mushroom growth. Onion peel waste was procured from the Nelpettai onion market, Madurai, India.

Mushroom cultivation using solid-state fermentation

The mushroom cultivation was carried out through solid-state fermentation. Initially, substrates were chopped into small pieces of particles that can enhance the cultivation process. The substrates were sterilized by autoclaving at 121°C at 15 lbs for 15 minutes. After sterilization, the substrates were cooled to room temperature before spawning. 2% of the spawn was used. A polypropylene bag (12cm x 18cm) was used for bed preparation. Temperature (28-35°C) and humidity (80-85%) were properly maintained throughout the cultivation period. Fruiting bodies of oyster mushroom originate from 10-14 days. Mushrooms were harvested based on flushing. After harvesting, the mushrooms were characterized by morphological features, nutritional and mineral contents.

Analysis of moisture in harvested mushrooms

Ten grams of freshly harvested mushrooms were taken into a sterile petri dish and dried in a hot air oven at 60°C (5) till a constant weight was reached. The moisture content was calculated as follows:

% Moisture = ((Initial weight of the mushroom sample-weight of the mushroom sample after drying)/ Initial weight of the sample) x 100

Analysis of ash content in harvested mushrooms

A known amount of each mushroom was taken in a crucible and kept in a muffle furnace at 600°C for 5-6 hours (5). Then the ash sample was cooled in a desiccator and weighed. Total ash content was estimated as follows:

Ash content = (Weight of ash/Weight of sample) x 100

Analysis of protein content in harvested mushrooms

Total protein in the mushrooms was estimated using the Folin-Phenol method. For this, 1 gram of mushroom was finely ground and mixed with 10ml of 0.1N NaOH and boiled for 30 minutes. Each mushroom sample was cooled to room temperature and centrifuged at 1200 rpm for 5 minutes. The supernatant was recovered, and total protein content was estimated based on Lowry et al. (6).

Analysis of total sugar content in harvested mushrooms

For this, 100 mg of each mushroom

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sample was hydrolyzed with 5ml of 2.5N HCl and was kept in a water bath for 3 hours at 100°C for further hydrolysis. The hydrolysate was brought down to room temperature and was neutralized using sodium carbonate. The neutralized sample was centrifuged at 1200 rpm for 10 minutes. The supernatant was recovered and analyzed for total sugars by the Anthrone method (7).

Analysis of lipid content in harvested mushrooms

Total lipids in the harvested mushrooms were extracted using the methanol-chloroform. For this, 2.5 grams of fresh mushrooms were ground and suspended in 25 ml of chloroform/ methanol (2:1 v/v). The solution was mixed well and allowed to settle for 3 days. After three days, the solution was transferred to a separating funnel and kept undisturbed for 10 minutes. The bottom phase was collected. After evaporation of chloroform, the sample was analyzed for lipid content (8).

Analysis of crude fiber content in harvested mushrooms

The crude fiber present in the mushrooms was estimated using volatilization gravimetric method (8). 2.5 grams of moisture and the lipid-free sample were taken and added with 200 ml of 0.255N sulfuric acid in a beaker. The solution was boiled for 30 minutes and filtered through filter paper. The filtrate was rinsed with hot water two to three times to remove acids. The filtrate was then transferred to the same beaker, and 200 ml of 0.313N NaOH was added, boiled for 30 minutes and filtered through a filter paper. The residue was washed with hot water to remove alkali. It was then transferred to a crucible and kept in the muffle furnace for 2 hours at 550°C.

$$\text{Crude fiber Content} = \frac{\text{Weight of the crucible with fiber- Weight of the crucible with ash)}}{\text{(Weight of the sample)}}$$

Analysis of mineral contents in harvested mushrooms

Mineral content present in the harvested mushrooms was analyzed using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS). For this, ash was digested with 2ml of concentrated nitric acid (HNO_3) and the digested solution was filtered through Whatman filter paper. The filtrate volume was made up to 50ml using deionized water and used for mineral analysis.

Results and Discussion

Harvesting of the first flush

Figures 1-6 show the mushroom cultivation using different substrates. Based on the substrate used, the harvesting time of mushrooms varied. Table 1 depicts the time for the first flushing of mushrooms cultivated using different substrates.

Table 1: Duration of Harvesting Mushrooms cultivated using Different Substrates

Substrates	Duration of harvesting (days)
Rice straw	27
Sugarcane	28
Groundnut shell	19
Sawdust (large)	22
Sawdust (small)	22
Onion Peel	25

Substrate Preparation

Bed Preparation

Harvesting Stage

Fig. 1 Cultivation of Mushrooms using Rice Straw as Substrate

Bed Preparation

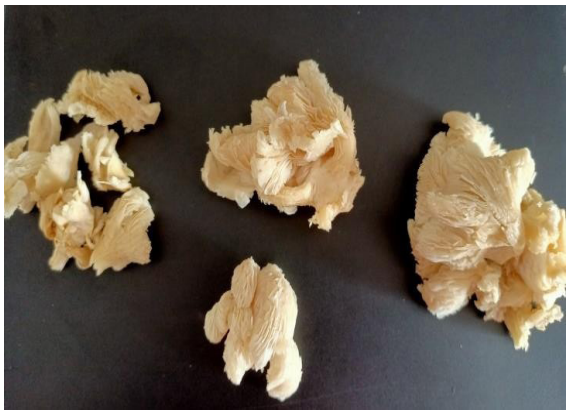
After 15 Days

Harvested Mushrooms

Fig. 2 Cultivation of Mushrooms using Sugarcane Bagasse as Substrate

Substrate Preparation

Harvesting Stage



Harvested Mushrooms

Fig. 3 Cultivation of Mushrooms using Groundnut Shell as Substrate

Substrate Preparation

Bed Preparation

Harvesting Stage

Fig. 4 Cultivation of Mushrooms using Sawdust (large particle) as Substrate

Substrate Preparation

Harvesting Stage

Fig. 5 Cultivation of Mushrooms using Sawdust (small particle) as Substrate

Bed Preparation

Bed Preparation

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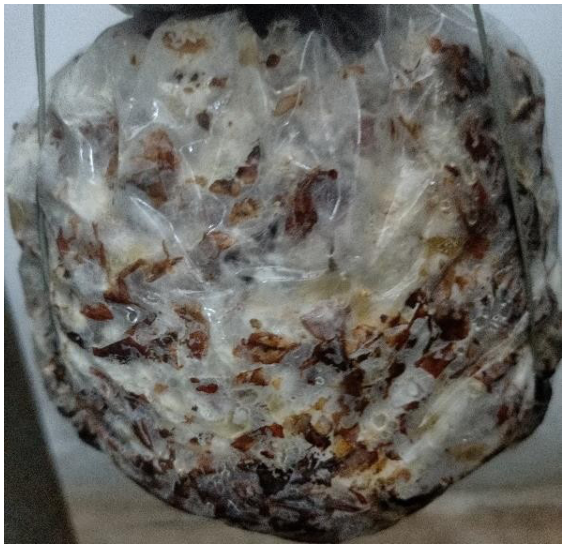

After 15 Days

Harvesting Stage

Fig. 6 Cultivation of Mushrooms using Onion Peel Waste as Substrate

Morphological parameters of harvested mushrooms

The cultivated *Pleurotus florida* was analyzed for morphological parameters. The results are tabulated in Table 2. As evident from the results, there were no significant changes in the stipe diameter for mushrooms cultivated using different substrates. A lesser number of fruiting bodies were observed in mushrooms grown in sawdust (small particles). The pileus diameter of mushrooms grown using sugarcane and rice straw was higher compared to mushrooms from other substrates. The length of the stipe for mushrooms did not vary significantly among all harvested mushrooms.

Table 2: Comparison of Morphological Parameters of Mushrooms Cultivated using Different Substrates

Substrates	Morphological Parameters			
	Number of fruiting bodies	Pileus diameter (cm)	Length of Stipe (cm)	Stipe diameter (cm)
Rice straw	4	6	3	1
Sugarcane bagasse	4	6.5	3	1
Groundnut shell	4	5	2.5	1
Sawdust (large)	4	4.5	3	1
Sawdust (small)	3	4.5	3	1
Onion peel	3	5.5	3	1

Impact of different substrates on the nutritional and mineral profile of *Pleurotus florida:* a comparative analysis

Nutritional analysis of harvested mushrooms

The nutritional labeling of different mushrooms harvested is depicted in Table 3. The yield of the mushroom grown using sugarcane bagasse was higher than the yield from other substrates used in this study. The moisture content of the cultivated mushroom was found to be higher, with groundnut shells as substrate. Mushrooms that were grown using rice straw as a substrate had higher carbohydrate content, while the protein content was higher in the mushrooms cultivated using sawdust of small particle size. Finally, the lipid content of the mushroom was lower in the rice straw, and crude fiber was higher when cultivated using sugarcane bagasse as substrate.

Table 3: Nutritional Content of the Mushrooms Cultivated using Different Substrates

Substrates	Yield of first flush (g/kg of substrate)	Moisture (%)	Ash (%)	Total Carbohydrates (g/100g)	Protein (g/100g)	Lipid (g/100g)	Crude fiber (g/100g)
Rice straw	57.25	65.5	5.23	17.56	1.065	4.76	7.0
Sugarcane bagasse	108.85	79.1	2.69	17.11	1.412	9.08	7.2
Groundnutshell	75.32	92	3.84	5.027	1.753	10.4	5.92
Sawdust (large)	48.37	84.2	3.92	2.725	4.57	12.08	6.4
Sawdust (small)	55.09	83.5	4.61	4.475	1.837	6.4	3.6
Onion peel	48.55	84.9	3.07	4.489	2.8	7.2	2.8

Mineral content analysis of harvested mushrooms

Table 4 depicts the mineral content (Ca, Mg, Na, K, Fe, Zn, Mn, Cu, and Se) of different mushrooms as assessed using ICP-MS. As seen from the table, mushroom cultivated using rice straw has higher zinc content while mushroom cultivated using sugarcane bagasse contain high levels of Mg, Na, Fe, Zn, M and Cu. In all the mushrooms, the selenium level was below the detection limit while the nickel content was below the detection limit for mushrooms cultivated using rice straw and sugarcane bagasse. This shows that the mineral levels in the cultivated mushrooms varied significantly based on the substrates used due to the bioaccumulation potential of mushrooms.

Table 4: Mineral Content of Mushrooms Cultivated using Different Substrates

Sl. No	Substrate	Mineral Content (mg/100g of mushroom)									
		Mg	Na	K	Ca	Fe	Zn	Mn	Cu	Ni	Se
1	Rice straw	13.85	20.9	210.7	1.915	0.39	6.00	0.06	0.12	BDL	BDL
2	Sugarcane bagasse	72.6	65.6	1442.6	2.0	4.08	2.96	0.6	0.83	BDL	BDL
3	Sawdust (Large)	33.45	39.75	577.45	1.92	1.99	1.81	0.17	0.39	0.028	BDL
4	Sawdust (Small)	31.3	39.45	685.45	7.4	3.25	1.355	0.2	0.215	0.03	BDL
5	Groundnut shell	65.85	59.6	2278.4	6.3	2.5	3.25	0.235	0.665	0.02	BDL
6	Onion peel	46.75	34.95	1464.1	6.2	3.25	1.715	0.255	0.35	0.02	BDL

BDL - Below detection level

Discussion

The substrates employed for mushroom cultivation differ in their chemical composition and mineral contents. Hence the quality and type of substrate may considerably affect the growth, morphological parameters and biochemical composition of cultivated mushrooms (9). Various nitrogen and carbohydrate-rich supplements have been found to have a significant impact on mushroom output and quality (10). In a study reported by Ogundele et al., (11), the total protein content, ash, crude fiber, crude fat and growth of *Pleurotus ostreatus* varied with different substrate combinations. However, no significant alteration was conveyed in total mineral composition. Studies indicated that different substrate combinations formulated with sawdust, corncob, and sugarcane bagasse presented a substantial variation in total colonization time, fruiting body characteristics, yield, biological efficiency, nutritional and mineral contents of *Pleurotus ostreatus* and *Pleurotus cystidiosus* (12). The combination of perilla stalks and cottonseed hulls used as an alternative substrate for the cultivation of *Pleurotus ostreatus* considerably enhanced the growth rate, productivity, biological efficiency, and nutritional composition in addition to the shortening of the cultivation cycle. Mushrooms cultivated on perilla stalks alone increased the amino acid content and antioxidant (13). Onyeka et al., (14) reported the differences in the nutrient composition of *Pleurotus ostreatus* mushroom using different substrates. Mushrooms harvested from substrate formula, sawdust + rice bran + $CaCO_3$ contained higher content of vitamins and minerals compared to other formulas. Harvest from cassava peels had the lowest protein content. The mushroom's nutritional value was improved through the application of nano-amino (Lithovit-Amino25) at spawning. The protein content increased at the highest dose while the essential amino acids increased at the lowest dose (15). It was reported that different substrates such as cassava peels, coconut residue, coffee waste and sawdust did not significantly affect the morphological features of *P. ostreatus*. Moreover, sawdust showed the lowest percentage of contamination as compared to others (16). A recent study has reported that the rice bran + saw dust combination used for *Pleurotus ostreatus* cultivation provided a higher yield in addition to carbohydrate sodium, chlorine and phytate contents when compared to ground banana leaves. However, the protein, phosphorus, vitamins B1, D and oxalate contents of mushrooms were considerably higher with ground banana leaves (17).

In the present study, *Pleurotus florida* was grown using different substrates such as rice straw, sugarcane bagasse, sawdust, groundnut shell and onion peel wastes. The results observed were concurrent with the reported work where the nutritional and mineral contents depend on the type and nature of substrate used. In this perspective, the mineral levels and the phytochemicals can be enhanced by using a particular type of substrate that is rich in certain elements or nutrients, thereby paving a way for the fortification of mushrooms through bioprocessing.

Conclusion

In this study, oyster mushroom, *Pleurotus florida* was cultivated using different substrates such as rice straw, sugarcane bagasse, sawdust, groundnut shell and onion peel wastes. To our knowledge, this is the first report stating the application of onion peel waste as a sole substrate for cultivation of oyster mushrooms. No significant alterations in the morphological features were observed in the mushrooms cultivated using different substrates. However, the nutrient contents of the cultivated mushrooms varied significantly with the type of substrate used. Further, it is observed that the particle size of the substrate (sawdust) influences the yield and nutritional content. The duration of the harvesting also varied with the substrates. Based on the analysis, sugarcane bagasse gave a better yield of mushrooms and mineral content differed with mushrooms harvested using different

mushrooms. Hence it can be concluded that the substrate not only plays a major role in influencing the yield of mushrooms through solid-state fermentation but also have an influence on the nutritional and mineral levels of the mushrooms. Hence, this bioprocessing strategy by varying the substrates for mushroom cultivation can be promising alternative for fortifying mushrooms with minerals essential to human health.

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Statements & declarations

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Author Contributions

Nithish Pandiyarajan, Barath Varadharajan and Vanavil Balakrishnan contributed to the study conception and design. Nithish Pandiyarajan, Barath Varadharajan and Nivethidha Karuppasamy performed the experiments. Nithish Pandiyarajan, Barath Varadharajan and Vanavil Balakrishnan analysed the results. The manuscript was written by Nithish Pandiyarajan and Vanavil Balakrishnan with input from all authors. All authors read and approved the final manuscript.

Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest.

Data Availability

All data generated or analyzed during this study are included in this manuscript

Ethics approval

Not Applicable

Consent to participate

Not applicable

Consent for publication

The authors give the consent for publication.

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