

Impact of varied substrates on the nutritional and yield aspects of *Pleurotus ostreatus* (Jacaum, ex. Fr. Kummer)

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ABSTRACT

Pleurotus ostreatus, commonly known as the oyster mushroom, is recognized globally for its nutritional and therapeutic properties. In this study, we aimed to investigate the nutritional and yield aspects of *P. ostreatus* when cultivated on different substrates. These substrates included rice straw, maize straw, wheat straw, soybean straw, chickpea straw, wood chips, sawdust, and a combination of wheat straw with full spawn inoculation. Among the substrates examined, wheat straw exhibited the highest moisture content (94.20%), surpassing all other substrates. Notably, the combination substrate of maize straw 25% with wheat straw 75% displayed the highest ash content (9.44%). Wood chips 100% exhibited highest flushes whereas Wheat straw 100% showed highest biological efficiency (%) and percentage increase in yield over control. The combination substrate yielded the lowest overall nutritional content compared to the other substrates. Remarkably, *P. ostreatus* cultivated on 100% rice straw exhibited the highest protein content (25.13%).

Keywords: *Pleurotus ostreatus*, maize straw, Agriculture wastes, Nutritional composition, yield

INTRODUCTION

Mushrooms are globally esteemed as a valuable food source and supplement due to their exceptional taste, nutritional richness, and potential health benefits. Chang in 1996 unequivocally confirmed mushrooms' pivotal role in human health, nutrition, and disease prevention. These macro-fungi exhibit distinctive fruiting bodies that are visible to the naked eye and can be hand-picked. They may emerge either above the ground (epigeous) or below it (hypogeous) and come in various sizes (Miles and Chang, 1997). Following *Agaricus bisporus*, *Pleurotus ostreatus*, commonly known as the oyster mushroom, stands as the second most cultivated edible mushroom globally (Sanchez, 2009). Originally cultivated in Germany to address nutritional needs during World War I (Eger *et al.*, 1976), oyster mushrooms, belonging to the Pleurotaceae family, have gained recognition for their safe and widespread consumption. These saprophytic organisms primarily thrive on woody logs and agricultural by-products, including sawdust, paper, pulp, straw, cornhusks, banana leaves, and other agricultural residues. *Pleurotus ostreatus* represents one of the 40 known species within the *Pleurotus* genus and is commonly referred to

as the "oyster mushroom". It thrives in tropical and subtropical regions and is readily cultivable (Deepalakshmi and Mirunalini, 2014). *P. ostreatus* features a broad, fan- or oyster-shaped cap, ranging from 5–25 cm in diameter. Natural specimens vary in colour, from white to grey, tan, or dark brown. When young, the cap's margin is rolled inward, later becoming smooth, often slightly lobed and wavy. Mushrooms, including *P. ostreatus*, are rich sources of protein, ash, lipids, crude fiber, carbohydrates, and various minerals (e.g., calcium, magnesium, sodium, phosphorus, potassium, sulphur, manganese, and iron). They also provide essential vitamins such as riboflavin, biotin, and thiamine (Chang and Buswell, 1996). Most edible mushrooms contain a substantial quantity of essential amino acids and water-soluble vitamins. The nutritional and medicinal properties of *Pleurotus* mushrooms have been extensively explored by researchers (Deepalakshmi and Mirunalini, 2014 Ao and Sharma, 2021). Some of these medicinal properties include anti-tumor effects linked to polysaccharides, antigenotoxic and bio-antimutagenic activities, anti-acidity, antihypertensive properties, and anti-hyperglycemic effects (Chang and Quimio, 1982). Mushrooms possess the remarkable

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ability to biodegrade organic matter and accumulate essential nutrients vital for human well-being. Therefore, cultivating *P. ostreatus* using various agricultural waste materials, such as sawdust, rice straw, and wood chips, can serve as an effective means of reducing the substantial quantities of these waste products. This approach holds the potential to address waste management challenges and environmental pollution. Additionally, the consumption of cultivated mushrooms can provide valuable nutritional benefits. Thus, this study aimed to cultivate *P. ostreatus* using diverse agricultural waste materials and analyse the proximate and mineral compositions of *P. ostreatus* fruit bodies cultivated on these different substrates.

MATERIALS AND METHODS

Substrate Preparation

The experiment comprised 25 different treatments, each with four replicates. These treatments were meticulously observed throughout the mycelium colonization and fruiting body formation stages, until the designated harvest time for subsequent analysis. The treatments encompassed a range of substrates, including rice straw, maize straw, wheat straw, soybean straw, chickpea straw, wood chips, sawdust, and a combination of all these substrates. To prepare the substrate bags for cultivation, they underwent sterilization in an autoclave at 121°C and 1.1 kg/cm² pressure for duration of 30 minutes. Following the sterilization process, the bags were left to cool overnight on a clean cupboard shelf.

Spawn Inoculation

The spawn bottles were procured 12 hours prior to the inoculation procedure and were stored in a controlled laboratory environment. Each substrate bag received a precise inoculation of 3.5 teaspoons of spawn material, equivalent to 30 grams of spawn per 1-kilogram bag of substrate. To ensure aseptic conditions, thorough cleaning and sterilization of the work area were conducted using absolute ethanol.

Incubation and Harvest

The substrate bags were stored in a controlled environment with a temperature maintained at 34°C and a relative humidity of 84%. These bags were allowed to undergo complete mycelium colonization undisturbed. Once the mycelium colonization was successfully completed, the mushroom bags were meticulously transferred to a screened greenhouse to initiate the fruiting phase. During this phase, regular irrigation was administered using a water sprayer, ensuring that the bags remained consistently moistened and ready for harvest. Subsequently, the mushrooms obtained from each substrate bag were harvested, their weights recorded, and they were subjected to air drying until a stable weight was attained. Following this drying process, the mushrooms were carefully placed into polythene bags and transported to the laboratory for a detailed analysis of their nutritional content and moisture composition.

Analysis and Determination of Nutritional Content

The proximate analysis and determination of protein contents in fruiting bodies from each substrate were carried out using standard methods. The Micro-Kjeldahl digestion and distillation procedure, as specified in AOAC (1935), were followed for nitrogen content determination, which served as the basis for calculating protein content.

$$\text{Nitrogen (\%)} = \frac{\text{Normality of H}_2\text{SO}_4 \times \text{Volume of 0.1N H}_2\text{SO}_4 \times 14}{\text{Weight of Sample} \times 1000} \times 10$$

$$\text{Crude Protein \%} = \text{Nitrogen\%} \times 6.25$$

The estimation of ash in the mushroom sample was conducted in accordance with the AOAC guidelines from 1984.

$$\text{Ash\%} = \frac{(\text{Initial weight of empty crucible and with sample}) - (\text{Final weight of Crucible with ash})}{\text{Weight of sample}} \times 100$$

Moisture content in each mushroom sample was independently determined using oven drying procedures.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight of the sample (g)} - \text{Dried weight of the sample (g)}}{\text{Sample weight (g)}} \times 100$$

The yield was determined using the following formula and reported in biological efficiency (%):

$$\text{Biological Efficiency (BE)} = \left(\frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \right) \times 100$$

Using Abbott's formula, the percent yield increase compared to the control was calculated (Abbotts 1925).

$$\text{Percent yield increased} = \frac{(T - C)}{C} \times 100$$

'C' Yield from control bags

'T' Yield of treated bags

RESULTS

Fruit body formation after 45 days of inoculation, wheat straw produced its initial flush of mushrooms, which were harvested the following day. Moisture is a critical factor for the normal biochemical processes in living organisms and also affects the storage quality of harvested plants. The moisture content of mature *P. ostreatus* fruit bodies harvested from various agricultural substrates ranged from 94.20% for wheat straw to 88.63% for soybean straw.

Table 1: Effect of different substrates on nutritional and yield aspects on *P. ostreatus*

Sr. no.	Treatments	Moisture (%)	Ash (%)	Protein (%)	1 st flush (days)	2 nd flush (days)	3 rd flush (days)	Biological efficiency (%)	% increase in yield over control
T ₁	Rice straw 100 %	93.125 ^{ab}	7.100 ^d	25.125	22.125 ^{klm}	36.675 ^{no}	52.125 ^{ij}	92.092 ^h	-7.658 ^h
T ₂	Rice straw 75% + 25% Wheat straw	93.350 ^{ab}	6.150 ^e	24.700 ^a	21.725 ^{mn}	36.275 ^{op}	51.725 ^j	97.120 ^a	-2.617 ^a
T ₃	Rice straw 50% + 50% Wheat straw	93.700 ^{ab}	4.408 ^{hjk}	24.625 ^a	21.400 ^{no}	35.725 ^{qr}	51.225 ^k	95.172 ^d	-4.565 ^d
T ₄	Rice straw 25% + 75% Wheat straw	93.875 ^{ab}	4.050 ^{kl}	23.200 ^{c e}	20.875 ^{pq}	35.350 ^{rs}	50.850 ^{kl}	93.145 ^f	-6.600 ^f
T ₅	Soybean straw 100 %	88.625 ^g	8.000 ^c	23.500 ^{bc}	24.150 ^f	39.650 ^h	54.150 ^e	83.805 ^m	-15.965 ^m
T ₆	Soybean straw 75% + 25 % Wheat straw	90.275 ^{ef}	6.995 ^d	23.300 ^{cde}	23.650 ^g	39.150 ^{ij}	53.900 ^e	95.105 ^d	-4.632 ^d
T ₇	Soybean straw 50% + 50 % Wheat straw	91.275 ^{def}	6.288 ^e	22.675 ^{fg}	22.950 ^h	38.450 ^k	52.925 ^{gh}	90.155 ⁱ	-9.598 ⁱ
T ₈	Soybean straw 25% + 75 % Wheat straw	91.600 ^{cde}	4.550 ^{hij}	22.275 ^{ij}	21.800 ^{mn}	37.300 ^m	51.825 ^j	79.695 ⁿ	-20.088 ⁿ
T ₉	Chickpea straw 100%	91.275 ^{def}	5.150 ^f	22.825 ^f	25.300 ^e	41.300 ^f	55.325 ^d	84.447 ^l	-15.320 ^l
T ₁₀	Chickpea straw 75% + 25 % Wheat straw	92.675 ^{bcd}	4.725 ^{gh}	22.650 ^{gh}	24.200 ^f	40.200 ^g	54.200 ^e	96.138 ^c	-3.600 ^c
T ₁₁	Chickpea straw 50% + 50 % Wheat straw	92.975 ^{abc}	4.325 ^{hijk}	22.625 ^{gh}	22.750 ^{hi}	38.650 ^k	52.650 ^h	92.955 ^g	-6.790 ^g
T ₁₂	Chickpea straw 25% + 75 % Wheat straw	93.600 ^{ab}	4.175 ^{ijkl}	22.375 ^{hij}	21.875 ^{lm}	37.875 ^l	51.875 ^j	89.678 ^j	-10.078 ^j
T ₁₃	Saw dust 100%	90.700 ^{ef}	5.075 ^{fg}	21.075 ^m	30.200 ^a	46.700 ^a	61.175 ^a	68.625 ^t	-31.188 ^t
T ₁₄	Saw dust 75% + 25 % Wheat straw	91.300 ^{def}	4.675 ^{ghi}	21.300 ^{lm}	27.350 ^c	43.850 ^c	58.350 ^b	78.485 ^o	-21.300 ^o
T ₁₅	Saw dust 50% + 50 % Wheat straw	92.800 ^{abc}	4.275 ^{ijk}	21.425 ^{kl}	25.400 ^e	41.900 ^e	56.400 ^c	75.560 ^p	-24.232 ^p
T ₁₆	Saw dust 25% + 75 % Wheat straw	93.700 ^{ab}	3.775 ^l	21.700 ^k	22.275 ^{kl}	38.800 ^{jk}	53.300 ^{fg}	71.668 ^r	-28.135 ^r
T ₁₇	Wood chips 100%	90.150 ^f	2.065 ^m	20.125 ^o	33.300	50.300	63.300	65.560 ^u	-34.260 ^u
T ₁₈	Wood chips 75% + 25 % Wheat straw	91.400 ^{def}	1.678 ^{mn}	20.675 ⁿ	28.125 ^b	45.125 ^b	58.125 ^b	79.750 ⁿ	-20.032 ⁿ
T ₁₉	Wood chips 50% + 50 % Wheat straw	92.600 ^{bcd}	1.450 ⁿ	21.300 ^{lm}	26.125 ^d	43.125 ^d	56.125 ^c	73.365 ^q	-26.438 ^q
T ₂₀	Wood chips 25% + 75 % Wheat straw	93.700 ^{ab}	1.315 ⁿ	21.700 ^k	22.475 ^{ijk}	39.475 ^{hi}	52.475 ^{hi}	69.970 ^s	-29.840 ^s
T ₂₁	Maize straw 100%	92.550 ^{bcd}	7.972 ^c	23.625 ^b	23.400 ^g	38.400 ^k	53.400 ^f	90.108 ⁱ	-9.648 ⁱ
T ₂₂	Maize straw 75% + 25 % Wheat straw	92.475 ^{bcd}	8.400 ^b	23.500 ^{bcd}	22.575 ^{hij}	37.575 ^{lm}	52.575 ^{hi}	96.292 ^b	-3.445 ^b
T ₂₃	Maize straw 50% + 50 % Wheat straw	93.375 ^{ab}	8.990 ^a	22.750 ^{fg}	21.850 ^{lm}	36.875 ⁿ	51.875 ^j	94.920 ^e	-4.820 ^e
T ₂₄	Maize straw 25 % + 75 % Wheat straw	93.750 ^{ab}	9.442	22.475 ^{ghi}	21.100 ^{op}	36.100 ^{pq}	51.100 ^{kl}	88.625 ^k	-11.132 ^k
T ₂₅	Wheat straw 100 %	94.200 ^a	7.275 ^d	22.150 ^j	20.600 ^q	35.100 ^s	50.600 ^l	99.727	0.000
	SE (m) ±	0.42	0.13	0.09	0.14	0.14	0.15	0.03	0.03
	CD _{p≥0.05}	1.18	0.39	0.27	0.40	0.40	0.42	0.09	0.09

The table below illustrates that the highest moisture content was found in wheat straw (94.20%), followed by rice bran 25% + 75% wheat straw (93.88%) and maize straw 25% + 75% wheat straw (93.75%). The lowest moisture content was observed in T5 - Soybean straw 100% (88.63%).

The protein and ash content in mature *P. ostreatus* fruit bodies grown on different agricultural substrates fell within the range of 25.13% to 20.13% for protein and 9.44% to 1.32% for ash content, respectively. The results demonstrated that the various agricultural wastes produced *P. ostreatus* with the highest protein content when cultivated on rice straw 100% (25.13%) substrate and the highest ash content on maize straw 25% + 75% wheat straw (9.44%). Conversely, the lowest protein and ash content were observed in wood chips 100% (20.13%) and wood chips 25% + 75% wheat straw (1.32%). This indicates that *P. ostreatus* could serve as a valuable protein source for incorporation into various products, including complementary foods. Wood chips 100% exhibited highest flushes and lowest in Wheat

straw 100%. Wheat straw 100% showed highest biological efficiency (%) and Percentage increase in yield over control whereas lowest in Wood chips 100%.

A correlation matrix was performed to understand the association among eight measured traits (Table 2). Moisture (%) showed a significant and negative correlation ($p \leq 0.05$) with 1st and 3rd flush (days) (-0.64* and -0.63*). Ash (%) showed a significant and positive correlation ($p \leq 0.05$) with biological efficiency (%) and Percentage increase in yield over control (0.63* and 0.63*). Protein (%) showed a significant and positive correlation ($p \leq 0.05$) with biological efficiency (%) and Percentage increase in yield over control (0.75* and 0.75*) where as significant and negative correlation with flushes. All flushes were showed a significant and negative correlation with biological efficiency (%) and Percentage increase in yield over control. Biological efficiency (%) showed a significant and positive correlation ($p \leq 0.05$) with Percentage increase in yield over control. Similar results reported by Shim *et al.*, 2007; Bae *et al.*, 2013.

Table 2: Correlation among eight measured traits

	Moisture (%)	Ash (%)	Protein (%)	1 st flush (days)	2 nd flush (days)	3 rd flush (days)	Biological efficiency (%)	% increase in yield over control
Moisture (%)	1.00							
Ash (%)	0.00	1.00						
Protein (%)	0.23	0.57	1.00					
1 st flush (days)	-0.64*	-0.45	-0.67*	1.00				
2 nd flush (days)	-0.61	-0.53	-0.75*	0.99*	1.00			
3 rd flush (days)	-0.63*	-0.44	-0.69*	0.99*	0.98*	1.00		
Biological efficiency (%)	0.35	0.63*	0.75*	-0.69*	-0.76*	-0.71*	1.00	
Percentage increase in yield over control	0.35	0.63*	0.75*	-0.69*	-0.76*	-0.71*	0.99*	1.00

The proximate composition of *Pleurotus ostreatus* samples cultivated on various substrates revealed distinct nutrient compositions. Moisture content in *P. ostreatus* samples grown on wheat straw 100%, rice straw 25% + 75% wheat straw, and maize straw 25% + 75% wheat straw combinations was determined as 94.20%, 93.88%, and 93.75%, respectively. Notably, the wheat straw treatment yielded mushrooms with the highest moisture content at 94.20%. This observation is consistent with earlier research indicating that mushroom moisture content is influenced by factors such as harvest timing, maturation

period, and environmental conditions during growth (Onyeka *et al.*, 2018; Elattar *et al.*, 2019; Akter *et al.*, 2022; Chaudhary, 2024). These variables likely account for the variations in moisture content among *P. ostreatus* samples from different substrate treatments.

Protein is a fundamental source of amino acids and a crucial dietary component (Hassan *et al.*, 2011). The crude protein content in samples from treatments involving rice straw 100%, rice straw 75% + 25% wheat straw, and rice straw (50% + 50% wheat straw substrates was determined to be 25.13%, 24.70%, and 24.63% per 1g of mushroom, respectively

Research suggests that mushroom protein content varies due to genetic factors of the species and the physical and chemical properties of the growth medium (Sanmee *et al.*, 2003; Roy *et al.*, 2015). Prior studies have indicated that any plant-based food contributing approximately twelve percent of its caloric value is considered a good source of protein. The present findings align closely with results from previous research, such as that conducted by Akter *et al.*, (2022), which concluded that rice straw is a superior substrate in terms of protein content. The quality of ash is associated with the presence of nutritionally significant mineral components in food materials (Olusanya, 2008). Regarding ash content, measurements were recorded as 9.44%, 8.99%, and 8.40% per 1g of mushroom for maize straw 25% + 75% wheat straw, maize straw 50% + 50% wheat straw, and maize straw 75% + 25% wheat straw substrate combinations, respectively. These findings are consistent with previous studies by Ahmed *et al.*, (2009) and Akter *et al.*, (2022), both of which suggested that maize straw outperforms other types of agricultural waste substrates in terms of ash content. Wheat straw 100% showed highest biological efficiency (%) and Percentage

increase in yield over control whereas lowest in Wood chips 100%. Therefore, amongst various substrates wheat straw bags perform better in both growth and yield of oyster mushroom.

CONCLUSION

In this research study, we conducted an investigation on the enhancing oyster mushroom (*Pleurotus ostreatus*) cultivation efficiency using different crop substrates for sustainable agriculture and waste management reduction. Remarkably, the combination substrate yielded the lowest overall nutritional content compared to the other substrates. In conclusion, this research underscores that *P. ostreatus* cultivated on wheat straw had the highest moisture content, while 100% rice straw yielded the highest protein content and yield. Therefore, the utilization of these substrates is recommended to optimize the nutritional benefits in the cultivation of *Pleurotus ostreatus*

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