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Evaluation of Yield Performance and Proximate Composition of *Pleurotus Tuber-Regium* (Ero usu) Grown on Different Substrates

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ABSTRACT

Pleurotus tuber-regium is a tropical mushroom of significant economic, cultural, and culinary importance in West Africa. Its ability to grow on a wide range of substrates may contribute to variations in yield and nutritional composition. Therefore, this study aimed to evaluate the yield performance and proximate composition of *P. tuber-regium* cultivated on different substrates. The substrates used were oil palm fiber (OPF), plantain leaves (PL), cassava tuber peels (CTP), and cocoa pod (CP). Substrate preparation, yield determination, and proximate analysis were conducted using standard procedures. The results showed that the highest yield was obtained from the CP substrate, whereas the lowest yields were recorded for OPF and PL. The highest moisture, ash, and fat contents were recorded in mushrooms cultivated on OPF, PL, and CP, respectively. The carbohydrate contents of *P. tuber-regium* cultivated on OPF, PL, CTP, and CP did not differ significantly ($p > 0.05$). Overall, the findings indicate that cocoa pod was the most suitable substrate, producing the highest mushroom yield and a favourable nutritional profile.

Keywords:
Pleurotus tuber-regium
Mushroom,
Proximate,
Yield and
Cocoa-pod

INTRODUCTION

Mushrooms are macrofungi that produce conspicuous, spore-bearing fruiting bodies and are valued worldwide as food, functional ingredients, and sources of bioactive compounds (Singh *et al*., 2025). Their vegetative phase consists of filamentous hyphae, which collectively form a mycelial network (Jo *et al*., 2023). -Under suitable environmental conditions, the mycelium differentiates into reproductive structures, including the fruiting bodies commonly recognized as mushrooms (Nowrousian, 2022). Although some mushroom species are poisonous, numerous edible species are widely consumed because of their nutritional value, including appreciable amounts of proteins, dietary fibre, minerals, vitamins, and other bioactive compounds (Assemie and Abebe, 2022; Lesa *et al*., 2022; Timm *et al*., 2025). Among the commercially important edible mushrooms, species of the genus *Pleurotus* are particularly notable because of their nutritional quality, relatively rapid growth, and capacity to utilize diverse lignocellulosic agricultural residues as cultivation substrates (Wal *et al*., 2023).

Among the *Pleurotus* species, *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer is a distinctive edible white-rot basidiomycete characterized by its ability to produce both fruiting bodies and a conspicuous sclerotium, a compact storage structure formed from aggregated fungal tissue (Sun *et al*., 2022). The species is distributed principally across tropical and subtropical regions and is capable of growing on a variety of lignocellulosic materials because of its wood-decaying ability (Aswathy *et al*., 2024). In Nigeria, *P. tuber-regium* is culturally and nutritionally important, particularly in southeastern communities, where both the fruiting bodies and sclerotia are consumed and the sclerotium, locally known as "usu" or "ero usu," is traditionally incorporated into food preparations. Recent

investigations have further demonstrated that the species possesses appreciable nutritional value and bioactive potential, supporting its importance as both a food mushroom and a potential functional-food resource (Aswathy *et al*., 2024).

The nutritional, functional, and economic importance of edible mushrooms has contributed to increasing interest in their cultivation (Jayaraman *et al*., 2024). Cultivation provides a more reliable means of production than dependence solely on wild populations and can improve the availability of nutritious mushroom products while creating opportunities for agricultural waste valorization and income generation (Mafe *et al*., 2025). This is particularly relevant in tropical Africa, where a wide diversity of agricultural residues is available for mushroom production and where *Pleurotus* species, including *P. tuber-regium*, are among the mushrooms most frequently cultivated (Timm *et al*., 2025). The ability of *Pleurotus* species to degrade lignocellulosic materials also makes them particularly suitable for converting low-value agricultural by-products into edible biomass and value-added food products.

The productivity and nutritional quality of cultivated mushrooms are influenced by several interacting factors, including mushroom species and strain, substrate composition, moisture content, pH, nutrient availability, environmental conditions, and cultivation practices (Timm *et al*., 2025). Of these factors, substrate composition is particularly important because it provides the carbon, nitrogen, minerals, and structural components required for mycelial growth and fruit-body development (Nuralykyzy *et al*., 2025). Differences in substrate composition can consequently affect mycelial colonization, biological efficiency, yield, and the proximate and mineral composition of harvested mushrooms (Timm *et al*., 2025). Recent evidence specifically demonstrates that the type and physicochemical characteristics of agricultural residues used as substrates can produce substantial differences in the yield and nutritional composition of *P. tuber-regium* fruiting bodies (Aswathy *et al*., 2024). Thus, appropriate substrate selection is essential for maximizing both production efficiency and nutritional quality.

Although *P. tuber-regium* has been successfully cultivated on several agricultural and lignocellulosic residues, comparative information remains limited for some locally abundant Nigerian agro-residues, particularly oil palm fiber (OPF), plantain leaves (PL), cassava tuber peels (CP), rice straw (RS), and cocoa pod husks. Previous studies have demonstrated the capacity of *P. tuber-regium* to utilize diverse agricultural residues, while recent work has confirmed substantial substrate-dependent differences in its growth, yield, biological efficiency, and nutritional composition (Batubenga *et al*., 2021; Aswathy *et al*., 2024). In tropical Africa, the availability of locally sourced agricultural by-products represents an important opportunity for expanding mushroom production while simultaneously contributing to sustainable waste management and circular bioeconomy practices (Timm *et al*., 2025). However, the relative suitability of OPF, PL, CP, RS, and cocoa pod husks for the cultivation of *P. tuber-regium* requires further empirical evaluation. Therefore, this study was undertaken to evaluate the yield performance and proximate composition of *P. tuber-regium* cultivated on these agricultural residues and to identify the substrate that provides the most favourable conditions for efficient and sustainable production (Aswathy *et al*., 2024).

MATERIALS AND METHODS

Study site

This study was conducted in a home garden located within the staff quarters of Akanu Ibiam Federal Polytechnic Unwana, Afikpo, Ebonyi State (latitude 5.8029298° N, longitude 7.9195051° E). The experiment involved five replications for each of the five treatments namely: oil palm fiber (OPF), plantain leaves (PL), cassava tuber peels (CTP), rice straw (RS) and cocoa pod (CP), arranged in a Completely Randomized Design (CRD).

Cultivation of Mushrooms on Agricultural Waste Substrates

Substrate preparation and pasteurization

Oil palm fiber (OPF), plantain leaves (PL), cassava tuber peels (CP), rice straw (RS), and cocoa pod (CPC) were used as substrates for mushroom cultivation. Each substrate was cut into pieces approximately 3–5 cm in length and soaked in water to achieve adequate hydration. The hydrated substrates were then subjected to steam pasteurization in a metal drum at approximately 90 °C for 20 min. Following treatment, the substrates were spread on clean plastic sheets and allowed to cool to below 25 °C before inoculation (Aswathy *et al*., 2024).

Bagging and spawn inoculation

After cooling, the substrates were transferred into transparent polyethylene bags under hygienic conditions, with 500 g of substrate placed in each bag. Wheat grain spawn of a single generation was incorporated at a rate of 10% of the substrate dry weight. The spawn was distributed in three alternating layers bottom, middle, and top to facilitate uniform colonization of the substrate. The open ends of the bags were tightly secured with clean rope. Small perforations were subsequently made in the bags using a sterilized needle to facilitate gaseous exchange. The inoculated

bags were then transferred to the mushroom production room and suspended randomly for the spawn-run period (Aswathy *et al*., 2024).

Spawn run and environmental conditions

During the spawn-run phase, the production room was maintained under dark conditions by covering the room with black polyethylene sheets. The temperature was maintained at approximately 25 °C, while relative humidity was maintained at 80–90%. No artificial illumination was provided during the spawn-run period. The bags were monitored regularly until the substrates were completely colonized by the white fungal mycelium. Complete colonization of the substrates marked the end of the spawn-run phase (Aswathy *et al*., 2024).

Fruiting induction and environmental management

Following complete colonization, the black polyethylene coverings were removed to permit the entry of dim or diffuse light, and adequate ventilation was provided to increase fresh-air exchange and facilitate fruiting initiation. The temperature was maintained at approximately 20 °C and relative humidity at about 90%. Humidity was maintained by periodic misting of the production-room floor and surrounding environment, taking care to avoid excessive direct wetting of the developing fruiting bodies (Aswathy *et al*., 2024).

Harvesting of fruiting bodies

Fruiting bodies were monitored regularly and harvested when the mushroom caps had developed fully but had not become excessively flattened or turned upward at the margins. Mushrooms were harvested carefully by gently twisting and pulling the fruiting bodies from the substrate while avoiding unnecessary damage to the colonized substrate. The remaining stubs were removed where necessary to minimize potential sites for contamination. Harvesting was continued for up to three flushes, with subsequent flushes allowed to develop following the appropriate interval between harvests (Kortei *et al*., 2018).

Determination of yield

The yield of mushrooms was assessed individually for each substrate bag, relying on the fresh weight of the fruiting bodies collected during both the first and second flushes. Upon harvesting, the fruiting bodies from each bag were meticulously cleaned to remove any substrate particles and then weighed using a calibrated electronic balance. The weight for each flush was documented separately in grams. The overall fresh mushroom yield per bag was then computed by adding the fresh weights recorded from the first and second flushes: (Aswathy *et al*., 2024). Total fresh yield (g/bag) = Fresh weight of first flush + Fresh weight of second flush.

Proximate Analysis

Moisture content determination

Precisely 20 g of fresh mushroom samples were weighed and dried in a hot-air oven at 105°C. After drying, the samples were cooled in a desiccator and reweighed. The process of heating, cooling, and weighing was repeated until a constant weight was obtained. The moisture content was determined according to AOAC (1980) and was expressed as a percentage and calculated using the following formula:

$$\text{Moisture} = \frac{wt\ of\ fresh\ sample - wt.of\ dry\ sample}{wt.of\ fresh\ sample} \times 100$$

Determination of ash content

Fibre content of the test samples was determined according to the AOAC method (1980). Exactly 2 g of the sample was placed in a beaker containing 1.2 mL of H_2SO_4 and boiled for approximately 30 minutes. The residue was then filtered, washed with hot water, and transferred to a beaker containing 1.2 g of NaOH per 100 mL of solution, which was boiled for about 30 minutes. The residue was washed again with hot water, dried in an oven and weighed (C2). The weighed samples were incinerated in a furnace at 550°C, removed, and allowed to cool before weighing (C3). The percentage fibre content of the cowpea seed sample was calculated using the formula below.

$$(\%)Ash = \frac{W2-W3}{W2-W1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Determination of mushroom sample fat content

The fat content of mushroom samples was determined following the method described by AOAC (1980). A clean, dry 250 mL boiling flask was placed in an oven, then transferred to a desiccator to cool. Filter paper was weighed and labeled (W1), and 2 g of the sample was weighed into thimbles (W2). The boiling flask was filled with n-hexane. The Soxhlet apparatus was assembled and allowed to reflux for 8 hours. Afterward, the flask was removed and placed in the oven to dry. Subsequently, it was transferred from the oven to the desiccator to cool before being weighed (W3). The percentage of fat content was calculated using the formula below.

$$(\%)Fat = \frac{W2-W3}{W2-W1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Determination of protein content of mushroom sample

Precisely 0.5 g of cowpea seed sample was weighed into a Kjeldahl flask. Two copper catalyst tablets were added, followed by 15 mL concentrated H_2SO_4. The resulting digest was steamed using the Markham distillation apparatus for 15 minutes. Under the condenser, a 100 mL

conical flask containing 10 mL of 4% (w/v) boric acid indicator was placed. Exactly 10 mL of the digest was introduced into the apparatus through the small funnel aperture. This was washed down with distilled water, followed by the addition of 10 mL of 40% NaOH solution. The solution was steamed for about 7 minutes to collect ammonium sulphate after which the receiving flask was removed, and the tip of the condenser was rinsed into the flask. The solution in the receiving flask was titrated with 0.01 N hydrochloric acid, and the nitrogen content was calculated, thereby determining the protein content of the sample. The percentage of protein was calculated using the formula below (AOAC, 1980).

$$\%Protein = \frac{(b-a)\times 0.1\times 14.00\times 100\times 6.25/1000}{Ws} \quad \ldots\ldots\ldots (3)$$

Where a = Volume (ml) of 0.1 N H_2SO_4 used in blank titration

b = Volume (ml) of 0.1 N H_2SO_4 used in sample titration

Ws = Weight (g) of sample

14.00 = Atomic weight of sample

1000 = Conversion factor of mgN/100 g

6.25 = Protein nitrogen conversion factor

Determination of Seed Carbohydrate Content

Carbohydrates (nitrogen-free extract, NFE), representing sugars and starches in food, were calculated by difference, i.e., % carbohydrate = 100 - (% moisture + % ash + % protein + % fat) (AOAC, 1984).

Data analysis

The data generated were presented as Mean ± Standard Deviation utilizing SPSS (Version 23). The analysis was conducted using one-way Analysis of Variance (ANOVA). Mean differences were assessed using the Tukey Test. A p-value of less than 0.05 was deemed statistically significant.

RESULTS

***Pleurotus tuber-regium* yield**

The yield performance of *Pleurotus tuber-regium* cultivated on various substrates namely oil palm fibre (OPF), plantain leaves (PL), cassava tuber peels (CTP), rice straw (RS), and cocoa pod (CP) is presented in Figure 1. The results indicate no significant difference in yield between *P. tuber-regium* grown on OPF and PL. However, the yields from these two substrates were significantly lower than those recorded for *P. tuber-regium* grown on CTP. The yield on CTP was, in turn, significantly lower than that obtained from RS, while the yield on RS was significantly lower than that recorded for CP.

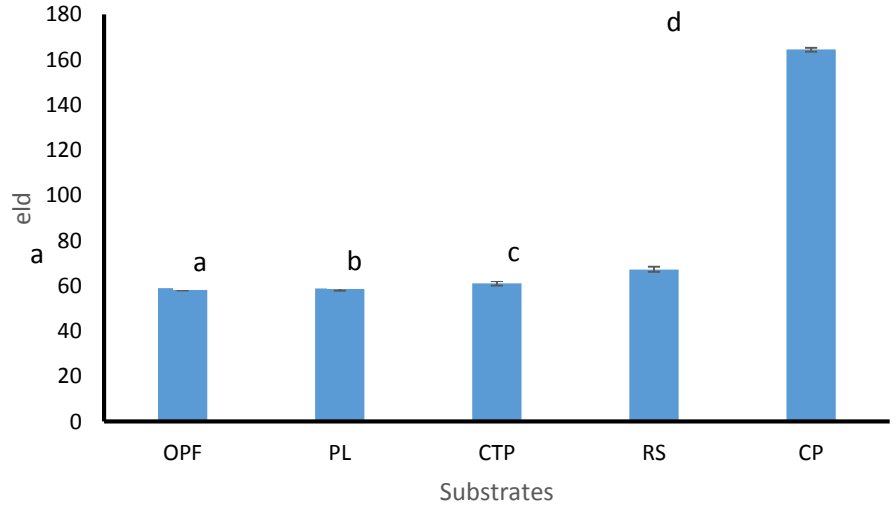


Figure 1: Yield performance of *Pleurotus tuber-Regium* on different substrate

Hints: OPF: [Oil palm fiber], PF: [Plantain leaves], CTP: [Cassava tuber peel], RS: [Rice straw], CP: [Cocoa pod]

Proximate composition

The proximate composition of *Pleurotus tuber-regium* cultivated on different substrates is presented in Table 1. The moisture contents of P. tuber-regium grown on RS, PL, and CP did not differ significantly from each other; however, they were significantly lower than those recorded for CTP and significantly higher than those recorded for OPF. The ash content of *P. tuber-regium* cultivated on OPF and CP did not differ significantly from each other but was significantly lower than that recorded for CTP. The ash content of CTP was not significantly different from that of RS but was significantly higher than that of PL. Similarly, the fat and protein contents of *P. tuber-regium* cultivated on OPF, PL, CTP, and RS did not differ significantly from one another but were significantly lower than those recorded for CP. The carbohydrate content of *P. tuber-regium* cultivated on OPF, PL, CTP, and RS did not differ significantly among these substrates but was significantly higher than that recorded for CP.

DISCUSSION

Yield performance is an important indicator of mushroom productivity because it reflects the quantity of fruiting bodies obtained from a given amount of cultivation substrate and, consequently, the effectiveness with which the substrate is converted into fungal biomass. In mushroom production, yield and biological efficiency are strongly influenced by substrate characteristics, including nutrient composition, carbon availability, lignocellulosic structure, moisture content, particle size, aeration and the accessibility of nutrients to the growing mycelium. *Pleurotus* species possess a well-developed lignocellulose-degrading system and can utilize a wide range of agricultural residues; however, their productivity varies substantially according to the type and composition of the substrate used (Ahmed *et al*., 2024). Recent studies have demonstrated significant differences in yield and biological efficiency when *Pleurotus* species are cultivated on different lignocellulosic residues, confirming that substrate selection is a major determinant of mushroom productivity (Biswas *et al*., 2023).

In the present study, *Pleurotus tuber-regium* cultivated on cocoa pod (CP) recorded the highest yield, indicating that CP provided comparatively favourable conditions for mycelial development, substrate colonization and subsequent fruit-body formation. The superior performance of *P. tuber-regium* on CP may be associated with the chemical composition of cocoa pod husk, which contains substantial quantities of structural carbohydrates together with appreciable amounts of lignin and mineral constituents. Recent characterization studies have reported that cocoa pod husk contains cellulose, hemicellulose and lignin as major structural components, together with a considerable ash fraction. For example, cocoa pod husk has been reported to contain approximately 24–35% cellulose, 8.7–11% hemicellulose and 14.6–23.4% lignin, although considerable variation occurs according to cultivar, geographical origin and processing conditions (Bugarin *et al*., 2025). Another recent chemical characterization reported 31.68% cellulose, 16.97% hemicellulose, 28.54% lignin and 9.24% ash in cocoa pod husk. These components provide a substantial carbon framework that can potentially be exploited by lignocellulose-degrading fungi.

The suitability of CP as a substrate may also be related to the ability of *P. tuber-regium* to secrete extracellular lignocellulolytic enzymes capable of breaking down complex plant polymers into simpler compounds that can subsequently be assimilated for fungal growth and fruit-body development (Ren *et al*., 2024). Aswathy *et al*. (2025) reported substantial differences in spawn-running time, primordia formation, yield, and biological efficiency of P. tuber-regium cultivated on different agro-waste substrates, with paddy straw yielding the highest mushroom production and biological efficiency among the substrates evaluated. The authors consequently emphasized that fruiting-body production is strongly dependent on the substrate used. Similarly, Biswas *et al*. (2023) observed significant differences in the yield and biological efficiency of *P. ostreatus* cultivated on different lignocellulosic residues, with rice straw producing considerably higher yield than several other substrates. These findings support the interpretation that the high yield obtained on CP in the present study may reflect a favourable combination of nutrient availability, structural carbohydrate composition and physical characteristics that enabled efficient substrate colonization and conversion into fruiting biomass.

The high yield obtained on CP is also consistent with the broader observation that *Pleurotus* species can efficiently convert agricultural residues into edible biomass, although the magnitude of conversion differs considerably among substrates. Ahmed *et al*. (2024) reported significant differences in the yield and biological efficiency of *P. ostreatus* cultivated on different combinations of rice straw, sawdust and waste tea leaves. Their findings showed that the addition of an alternative lignocellulosic material could either enhance or reduce yield depending on the substrate combination,

emphasizing that the mere presence of cellulose-rich material does not guarantee superior mushroom productivity. Thus, the present high yield on CP may have resulted from the interaction between its chemical composition and the physical conditions it provided for fungal growth rather than from the abundance of any single nutrient.

In contrast, the relatively lower yield recorded for *P. tuber-regium* cultivated on oil palm fibre (OPF), plantain leaves (PL), cassava tuber peel (CTP) and rice straw (RS) may be associated with differences in substrate composition, nutrient accessibility, moisture dynamics, lignocellulosic structure and the degree of resistance of structural polymers to enzymatic degradation. The ability of a substrate to support mushroom production depends not only on the total concentration of cellulose, hemicellulose and lignin but also on their relative proportions and accessibility to fungal enzymes. Recent research has demonstrated that different lignocellulosic substrates undergo distinct patterns of structural degradation during *Pleurotus* cultivation, which consequently influence yield and biological efficiency (Ahmed *et al*., 2024). Therefore, the lower yield obtained from OPF, PL, CTP and RS should not necessarily be interpreted as evidence of inadequate nutrient content; rather, it may indicate differences in nutrient accessibility and substrate suitability for this particular *P. tuber-regium* strain under the cultivation conditions employed.

This interpretation is supported by the recent study of Aswathy *et al*. (2024), in which *P. tuber-regium* exhibited markedly different production responses on paddy straw, sugarcane bagasse and sawdust. Paddy straw supported the highest yield of 211.90 g/kg substrate and biological efficiency of 98.59%, whereas lower values were recorded on sugarcane bagasse and sawdust. Interestingly, rice husk did not support fruiting, demonstrating that even closely related agricultural residues can differ considerably in their suitability for *P. tuber-regium*. Similarly, studies on *P. ostreatus* have reported substantial substrate-dependent differences in yield, further indicating that substrate composition and physical characteristics interact to determine mushroom productivity (Biswas *et al.*, 2023). The reduced yields observed on OPF, PL, CTP and RS in the present investigation may therefore reflect differences in the extent to which nutrients were accessible and efficiently converted into fruiting biomass.

The moisture content of the harvested *P. tuber-regium* varied among the substrates, demonstrating that cultivation substrate can influence the water status of the resulting fruiting bodies. The comparatively high moisture content recorded in mushrooms cultivated on cassava tuber peel (CTP) may be associated with differences in the water-retention properties of the substrate and the availability of water during mycelial growth and fruit-body development. Water is essential for fungal metabolism, nutrient transport, enzymatic activity and fruit-body formation; consequently, substrate moisture and water availability are important considerations in *Pleurotus* cultivation. Ahmed *et al*. (2024), for example, maintained cultivation substrates within controlled moisture ranges and demonstrated that changes in substrate formulation affected both biological efficiency and the biochemical characteristics of the resulting mushrooms.

The relatively high moisture content recorded in *P. tuber-regium* cultivated on CTP may also be related to differences in the physical characteristics and carbohydrate composition of cassava-derived residues. However, this explanation should be interpreted cautiously because the water-holding capacity of the individual substrates was not determined in the present study. Moreover, moisture content of the harvested mushroom represents not only water supplied by the substrate but also the physiological and structural characteristics of the fruiting body. Recent studies have demonstrated significant substrate-associated variation in the moisture content of *Pleurotus* fruiting bodies. In a study involving *P. ostreatus* cultivated on oil palm fibre, rice husk, sawdust, guinea grass, bean pods and rice straw, substantial differences were observed in moisture and other proximate parameters among substrates (2023). Likewise, Purane *et al*. (2023) reported significant variation in moisture content among *P. florida* cultivated on different agricultural wastes. These observations support the possibility that the higher moisture content observed in the CTP treatment was associated with substrate-dependent differences in moisture availability and fruit-body characteristics.

Conversely, the comparatively low moisture content recorded in *P. tuber-regium* cultivated on OPF may reflect differences in the physical structure, porosity and moisture-retention characteristics of oil-palm fibre. Oil palm residues are predominantly fibrous lignocellulosic materials, and their physical properties may influence water distribution and accessibility within the cultivation matrix. Nevertheless, the relationship between substrate and mushroom moisture content is complex, and a lower moisture content in the fruiting body cannot be attributed solely to reduced water-holding capacity of the substrate without direct measurements. Recent evidence nevertheless confirms that oil-palm-based substrates can produce *Pleurotus* fruiting bodies with proximate compositions that differ from those obtained on other

agricultural residues (Rungjindamai *et al*., 2024). Therefore, the relatively low moisture content recorded in mushrooms cultivated on OPF may reflect differences in substrate moisture dynamics and the characteristics of the resulting fruiting bodies.

Ash content represents the inorganic residue remaining after combustion and is therefore commonly used as an indirect measure of the total mineral fraction of food materials. In the present study, *P. tuber-regium* cultivated on plantain leaves recorded a comparatively lower ash content. This finding may be associated with differences in the mineral composition and availability of the plantain-leaf substrate relative to the other substrates. However, because the mineral composition of the original substrates was not determined in this investigation, it would be inappropriate to conclude that the lower ash content resulted specifically from a deficiency of minerals in plantain leaves. Instead, the finding suggests that differences in substrate mineral composition and mineral uptake may have contributed to the observed variation.

Ash content represents the inorganic residue remaining after the combustion of organic material and is commonly used as an estimate of the total mineral fraction of food materials. In the present study, P. tuber-regium cultivated on plantain leaves recorded comparatively lower ash content, suggesting that the substrate used may have influenced the mineral composition of the harvested fruiting bodies. This observation is consistent with Aswathy et al. (2024), who reported differences in the ash content of P. tuber-regium cultivated on different agro-waste substrates, with values of 5.14, 3.76, and 3.29 g/100 g for paddy straw, sawdust, and sugarcane bagasse, respectively. The authors also reported differences in mineral concentrations among the substrates and attributed these variations, in part, to differences in substrate composition and mineral availability.

Similarly, Timm *et al*. (2025) highlighted the influence of cultivation substrates on the nutritional and elemental composition of edible mushrooms, supporting the view that substrate type can contribute to variation in mushroom ash content. Nevertheless, because the mineral composition of the plantain leaves and other substrates was not determined in the present study, the lower ash content cannot be attributed conclusively to a lower mineral concentration in plantain leaves. Other factors, including differences in mineral bioavailability, fungal uptake, and the accumulation of inorganic constituents in fruiting bodies, may also have contributed to the observed variation.

Recent studies provide strong evidence that substrate composition can influence the mineral and ash contents of *Pleurotus* fruiting bodies. For example, cultivation of *P. ostreatus* on oil palm fibre, rice husk, sawdust, guinea grass, bean pods and rice straw resulted in significant differences in ash and mineral composition among the fruiting bodies (Rungjindamai *et al*., 2024). Ahmed *et al*. (2024) similarly demonstrated substrate-dependent variation in mineral accumulation in *P. ostreatus*. Importantly, their findings showed that the concentration of a mineral in the fruiting body did not always correspond directly to its concentration in the substrate, indicating that mushroom species and strains differ in their capacity to absorb and accumulate individual mineral elements. Therefore, the relatively low ash content observed in the PL treatment may reflect differences in mineral availability, uptake efficiency and physiological accumulation rather than simply the total mineral content of the plantain-leaf substrate.

In contrast, the comparatively higher ash contents observed in *P. tuber-regium* cultivated on CTP, RS, OPF and CP may indicate greater accumulation of inorganic constituents in the fruiting bodies under these cultivation conditions. Cocoa pod husk is particularly notable for its mineral-rich nature. Recent chemical characterization has reported ash values of approximately 9–11% in cocoa pod husk, together with substantial amounts of cellulose, hemicellulose and lignin. Such a mineral-rich residue could potentially contribute to the mineral fraction of mushrooms cultivated on CP. However, mineral uptake by mushrooms is selective rather than a simple passive transfer process. Ahmed *et al*. (2024), for example, found that some elements were accumulated by *P. ostreatus* at concentrations that exceeded those in the cultivation substrate, whereas other elements were not proportionally accumulated. Thus, the elevated ash contents observed in the present study should be interpreted as substrate-associated differences in mineral accumulation rather than direct proof of mineral transfer from the individual substrates.

The fat content of *P. tuber-regium* also varied according to the cultivation substrate. In the present study, the fat contents of mushrooms cultivated on OPF, PL, CTP and RS were lower than that obtained from CP. This finding suggests that substrate type may influence the proximate composition of the resulting fruiting bodies, although the mechanism underlying differences in lipid accumulation cannot be established from the present experiment alone. Mushrooms generally contain relatively low amounts of fat compared with many animal-derived foods, but their lipid composition and concentration may vary according to species, strain, developmental stage and cultivation substrate. Recent research has demonstrated substantial

differences in crude fat content among *Pleurotus* fruiting bodies cultivated on different agricultural wastes (Purane *et al*., 2023).

The higher fat content recorded in *P. tuber-regium* cultivated on CP may therefore be related to differences in the availability and assimilation of carbon and other nutrients provided by the substrate. Because cocoa pod husk contains substantial carbohydrate and structural polysaccharide fractions, its degradation could provide carbon substrates that are subsequently incorporated into fungal biomass and cellular metabolites. Nevertheless, the observed increase in crude fat should not be interpreted as evidence that CP directly stimulates lipid biosynthesis. Rather, it may reflect differences in nutrient assimilation and metabolic allocation during fruit-body development. This interpretation is consistent with the findings of Timm *et al.* (2025) who observed substrate-dependent differences in the proximate composition of *P. tuber-regium*, including fat content. Their study found that *P. tuber-regium* cultivated on paddy straw produced high yields and protein and carbohydrate contents but relatively low fat, demonstrating that high productivity does not necessarily coincide with high lipid accumulation.

The present observation is also consistent with recent work on *P. ostreatus*, where substrate selection significantly affected crude protein, crude fat, ash, moisture, carbohydrate, and mineral and antioxidant contents of the resulting fruiting bodies (Rungjindamai *et al*., 2024). Similarly, Purane *et al*. (2023) reported significant differences in the fat and ash contents of *P. florida* cultivated on different agro-wastes. Collectively, these findings demonstrate that the nutritional composition of *Pleurotus* fruiting bodies is not fixed but can be modified by the cultivation substrate. Therefore, the comparatively higher fat content obtained from CP in the present study may reflect the particular nutrient environment created by this substrate and its interaction with the metabolic characteristics of *P. tuber-regium*.

Overall, the present findings demonstrate that substrate type markedly influenced both the productivity and proximate composition of *P. tuber-regium*. Cocoa pod produced the highest yield and was associated with comparatively favourable nutritional characteristics, while the other substrates produced distinct patterns of moisture, ash and fat accumulation. These differences reinforce the importance of substrate selection in the cultivation of *P. tuber-regium*. Recent research consistently demonstrates that agricultural residues differ substantially in their ability to support *Pleurotus* growth, yield and nutritional quality, and that no single chemical component can fully explain substrate performance (Biswas *et al*., 2023; Aswathy *et al*., 2024; Ahmed *et al*., 2024). The superior performance of CP in the present study therefore appears to reflect the combined effects of its chemical composition, nutrient accessibility and physical characteristics. Consequently, cocoa pod represents a promising locally available lignocellulosic residue for *P. tuber-regium* cultivation and provides an additional opportunity for converting an underutilized agricultural by-product into nutritionally valuable edible biomass.

CONCLUSIONS

In conclusion, the results show that substrate type had a significant impact on both productivity and proximate composition of *P. tuber-regium*. The higher yield obtained with CP, together with changes in moisture, ash, and fat levels, suggests that cocoa pod is a potentially acceptable agricultural residual for the development of this mushroom. However, yield and nutritional quality are not always dictated by a single substrate component; rather, they are most likely determined by a combination of substrate chemical composition, moisture availability, physical structure, nutrient accessibility, fungal strain, and growing circumstances.

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