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The effects of *Pleurotus tuber-regium* solid-state fermentation on the fermentation and physical characteristics of selected biomass wastes

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Abstract

The fermentation and physical characteristics of biomass wastes (banana pseudo-stem, empty palm bunch, and rice hull) subjected to solid-state fermentation using the king tuber mushroom (*Pleurotus tuber-regium*) were investigated. Each of the biomass wastes were milled as necessary and composted for seven days and thereafter inoculated with the mushroom spores and fermented anaerobically to achieve mycelia colonization for the periods of 20, 30, and 40 days in triplicates in a completely randomized design. Data on mycelia colonization, color, odor, pH, and temperature changes were recorded every 10 days. Analysis of physical characteristics was performed on fermented and non-fermented biomass wastes. Results showed that *Pleurotus tuber-regium* colonized empty palm bunch and rice hull by the 20th day of fermentation but failed to colonize banana pseudo-stem. Mycelia colonization was visually observed to be more extensive on the substrate, with pin head development in empty palm bunch and rice hull by the 30th day, but died off by the 40th day. Fermentation temperature significantly decreased ($p < 0.05$) in all substrates, while pH of empty palm bunch and rice hull increased significantly ($p < 0.05$) from slightly acidic (6.16 and 6.82, respectively) to neutral (7.00). Physical characteristics of all biomass wastes revealed a significant decrease ($p < 0.05$) in treated biomass wastes values compared to control values. It was concluded that the 20-day fermentation period using *Pleurotus tuber-regium* on composted empty palm bunch and rice hull was optimal, based on the observed improvements in their physical characteristics. This makes them potential feedstuffs for ruminants and pseudo-ruminants.

Keywords: *Pleurotus tuber-regium*, Biomass wastes, Banana pseudo-stem, Empty palm bunch, Rice hull/husk

Introduction

The abundance of biomass wastes such as the banana pseudo-stem, empty palm bunch and rice hull have been documented at most locations in Nigeria [1]. These biomass wastes belong to farmers who could readily utilize them in feeding livestock or sell them to feed processors for extra income. Their utilization in animal feeding is however constrained by factors such as difficulty in gathering and transporting them, significant variations in their nutrient composition across batches, presence of anti-nutrients, fibrous nature and low nutrient availability [2, 3, 4, 5]. Amongst these constraints, the high fiber and low nutrient availability remain major concerns that need to be addressed in order to effectively incorporate them into livestock diets. Several technologies such as chemical, physical, mechanical and biological treatments have been used to improve the nutrient values of these materials for different livestock, with promising reports both at the research stations and in the field. Some of these techniques have not been readily adopted by farmers because of their costly inputs and hi-tech nature [6, 7].

White rot fungi such as some *Pleurotus* species are reported to be effective in improving the nutrient values of fibrous materials [8]. While several studies have documented mostly the use of rice hull in combination with other biomass materials as substrate for the production of the king tuber mushroom *Pleurotus tuber-regium* [9, 10]. Very few studies have combined EFB and other materials for the same purpose [11]. There is however no documented study on the use of banana pseudo-stem as substrate for *P. tuber-regium* production. Several studies have also shown the effects of mushroom cultivation on the physical properties of the substrate [12, 13, 14]. The observed changes can be attributed to the mushroom's ability to biodegrade the fiber components of the substrate [15]. There is also the need to understand the effect of short-term *P. tuber-regium* mycelial colonization of these substrates on their nutrient compositions. This is because the nutrient contents of spent substrate reported in most livestock nutrition studies have been shown to be partially depleted by the mushrooms, while bioactive enzymes produced during the biodegradation process may also have been lost [16].



Several studies have also reported the effects of *P. tuber-regium* mycelia colonization on the nutrient compositions of biomass wastes [15, 17 18], there are however no such published reports on the effects on physical properties such as bulk density, water holding capacity and oil absorption capacity which are known to influence the intake and utilization of feedstuff by animals [19]. Again, fermentation process is reported to affect other physicochemical characteristics of the substrate such as the colour, odour, temperature and Ph [20, 21, 22, 23]. These changes are usually the earliest indications of the success of the fermentation process and therefore need to be monitored.

The aim of this study is to determine the fermentation and physical characteristics of biomass wastes subjected to solid-state fermentation using the king tuber mushroom (*P. tuber-regium*).

Materials and methods

Study Location

The research was conducted at the Teaching and Research Farm and the Laboratory of the School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri (FUTO), Imo State, located in the Southeastern part of Nigeria. Imo State is situated in the rainforest vegetation belt of Nigeria and lies within latitudes 4°45'N and 7°15'N, and longitude 6°50'E and 7°25'E, and covers an area of about 5,100 sq km. It is bordered by Abia State in the East, River State in the west and south and Anambra State in the north. The area is dominated by plains of about 90 m above sea level except for elevations associated with the Okigwe high lands [24]. It has a tropical climate with two distinct seasons; a rainy season that runs from April to October and a dry season that lasts from November to March, with annual rainfall reaching 2,200 mm (80 inches). The average daily temperature ranges from 28 to 30°C, while the average humidity is about 75% reaching 90% in the rainy season. The dry season experiences two months of Harmattan from late December to late February.

Collection and preparation of the biomass wastes

Banana pseudo stem (BPS) was sourced from farms and house-holds within Owerri town and its environs. The BPS was collected fresh immediately after fruit harvest, then chopped into smaller pieces and sundried for 4 to 5 days. The empty palm-fruit bunch (EPB) was also sourced from within Owerri and its environs. It was washed with clean water to remove any debris or unwanted materials such as sand, and cut into smaller pieces, of about 1-2 cm in length and thereafter, sun-dried. The rice hull (RHL) was collected from a rice mill at Orji in Owerri North Local Government Area (LGA) of Imo State. A magnet was passed through the RHL to remove all metal fragments in it before further sorting to remove debris and other unwanted materials. The PBS and EPB were milled with a food grinder (Mastercheff® Blender, Model Mc - 211) to an appropriate particle size (slightly coarse) and stored in polyethylene woven sacks until it was used. Thereafter, 4.5 kg of each biomass waste was composted according to the procedures described by [25, 26]. They were moistened with borehole water at the ratio of one kilogram of material to two liters of water and mixed thoroughly to ensure complete wetness. The damp materials were subsequently piled up in heaps and covered with polythene sheets to create the anaerobic

conditions favorable for composting [27]. The heaps were turned daily in the mornings to ensure aeration and proper composting. After seven days the heaps were uncovered and allowed to cool.

Experimental design, mushroom preparation and inoculation

The tubers (sclerotia) of *P. tuber-regium* purchased from a local market in Owerri were washed thoroughly to eliminate any potential dirt. They were thereafter immersed in water for an hour and transferred to a transparent plastic bucket and covered properly for three days to allow for spore formation (spawn production). The spawned *P. tuber-regium* sclerotia were subsequently removed and sliced into smaller pieces carrying the spores, according to the method reported by [26]. Composted biomass waste was loaded onto wooden trays, which were constructed with 2" x 2" wood frames and wire mesh bottoms. The bottom of each tray was lined with white polyethylene sheet which was disinfected with methylated spirit and the composted biomass waste was placed on top of the polythene sheet.

The solid-state fermentation of the biomass wastes (EPB), (BPS) and (RHL) was for a period of 40 days divided into three stages of 20, 30 and 40 days. The composted materials were divided into four treatments (T1, T2, T3, and T4), with T1 being the unfermented biomass wastes serving as control. The T2 - T4 represented 20, 30 and 40 days respectively. Each of the biomass waste treatment was replicated three (3) times in a completely randomized design (CRD).

Spores of the *P. tuber-regium* were inoculated into the composted biomass waste at a ratio of 100 g of spores to 500 g of biomass waste. The polyethylene sheet was closed after inoculation to create anaerobic environment. The samples were placed in triplicates in an inoculation room for 20, 30, and 40 days to ensure that the composted biomass waste is completely colonized by the fungal mycelia, indicated by whitish growth. At the end of each period, the samples were taken out of the inoculation room and sun-dried to stop mycelial growth. The dried material was stored in polyethylene woven sacks at room temperature until needed for laboratory analysis.

Data collection

During the fermentation process, the moisture, temperature and pH value of the substrates were measured at 10 days intervals using a digital pH, temperature and moisture meter (Mitsubushi, Japan). More so, the colour, odour and percentage substrate colonization of the mycelia determined at days 20, 30 and 40 in triplicates. All measurements were carried out in triplicates.

Analysis of the physical characteristics of biomass wastes

This involved the determination of the bulk density (BD), water holding capacity (WHC), swelling capacity (SWC), oil absorption capacity (OAC) of the biomass wastes, and their fermented products using the methods described by [28].

Data analysis



The data generated from the study was subjected to Analysis of Variance (ANOVA) and the mean differences were compared using the Duncan's Multiple Range Test from the Statistical Package for Social Sciences (SPSS) User's Guide [29]

Results and Discussion

Fermentation characteristics of the biomass wastes

The results of the *P. tuber-regium* fermentation characteristics of the banana pseudo-stem (BPS), empty palm bunch (EPB) and rice hull/husk (RHL) are presented in tables 1 to 3. The physical changes in the substrates during the fermentation period as presented in table 1 showed that mycelia colonization by the *P. tuber-regium* occurred in the EPB and RHL by the 20th day of the fermentation. The *P. tuber-regium* however could not colonize the BPS throughout the 40 days fermentation period. In the EPB and RHL, increased mycelia colonization, with the development of pin heads were observed by the 30th day of fermentation although the pin heads dried off by the 40th day. The 20 – 30th days EPB and RHL changed from their original light brown to the whitish colour as a result of the mycelia colonization, although they reversed to brown colour by the 40th day, indicating probably the termination of mycelia development. The BPS emitted a characteristic ammonia odour of decreasing intensity with fermentation periods, while the EPB emitted a decaying wood odour all through. The RHL however emitted an alcoholic odour up to the 20th day before changing to decaying wood odour. The biomass composting method adopted in this study resulted in the production of significant amounts of ammonia gas by the substrates similar to the report of [20], and in response to the daily turning and wetting of the substrates to achieve their aeration [30, 31]. The ammonia content of the substrate is however expected to reduce by the end of the composting period [21], since higher than 0.07% ammonia concentration in the substrate will limit its colonization by mushrooms [32].

The continued perception of ammonia odour in the BPS during the early days of the fermentation therefore, indicates that seven days is not enough to achieve its proper composting for *P. tuber-regium* mycelia establishment.

It is therefore possible that the ammonia content of composted BPS exceeded the 0.10% threshold that would limit the *P. tuber-regium* growth. The change in the colour of the substrates from light to dark brown or chocolate-brown colour agrees with the reports of [21]. The development of pinheads and fruiting bodies in the EPB and RHL may not be ideal for this type of study since their development involves the uptake of nutrients from the substrate, and could therefore cause significant depletion of the nutrients in the substrates. The non-composting approach consisting of steam sterilization of the biomass waste before inoculation of the sclerotia, which is commonly used in commercial production of *P. ostreatus* spawn may therefore be more suitable for achieving the present objective [33], which is mycelial colonization of the substrate.

The result in table 2 highlights the changes in temperatures during the *P. tuber-regium* fermentation of the biomass wastes. There was a general decrease in temperature across biomass wastes, such that the 40th day values were significantly lower ($p < 0.05$) than the 20th day values. The significantly higher temperatures on the 20th day suggest that the fermentation activities were most active during that period resulting in elevated heat generation in the substrate. The statistical similarity between the 20th and 30th day values of the EPB however suggests that its fermentation activities sustained to the 30th day, contrary to that of the other substrates. The temperature range recorded in the present study is within the 20 – 30°C optimum temperature recommended for *P. tuber-regium* mycelia growth [34]. [22] however reported that the mushroom thrives best at 35°C.

Table 1: Physical changes observed during the fermentation of the biomass wastes

Parameters	Banana pseudo-stem (BPS)	Empty palm bunch (EPB)	Rice hull/husk (RHL)
Mycelia colonization	-ve	+ve	+ve
Visual observation of colonization	-ve	From 20 th day	From 20 th day
Development of pinhead	-ve	Intense colonization with pin head development at day 30 which died at day 40	Intense colonization with pin head development at day 30 which died at day 40. Fruiting body was formed between days 30 and 40
Colour	Changed from light to dark brown	Change from light brown to whitish due to fungal colonization at the 20 th – 30 th day, then changed to brown by 40 th day	Change from light brown to whitish due to fungal colonization at the 20 th – 30 th day, then changed to brown by 40 th day
Odour	Ammonia odour with decreasing intensity with fermentation period	Decaying wood odour all through the fermentation period	Alcoholic odour that changed to decaying wood odour by day 20 and extending throughout the fermentation period



The changes in the pH values of the biomass wastes during their *P. tuber-regium* fermentation are presented in table 3. There was a progressive increase in the pH of the

EPB and RHL such that the 40th day values were significantly higher ($p < 0.05$) than the 20th day values.

Table 2: Changes in the fermentation temperatures (°C) of *P. tuber-regium* treated biomass wastes

Biomass wastes	Fermentation periods (days)			SEM
	20 th day	30 th day	40 th day	
BPS	29.00 ^a	27.67 ^b	28.00 ^b	0.22
EPB	30.33 ^a	29.00 ^{ab}	28.33 ^b	0.36
RHL	29.33 ^a	28.33 ^b	28.00 ^b	0.13

^{abc} means with different superscript in a row are significantly different ($p < 0.05$), BPS = Banana pseudo-stem, EPB = Empty palm bunch, RHL = Rice hull/husk

Table 3: changes in the fermentation pH of *P. tuber-regium* treated biomass wastes

Biomass wastes	Fermentation periods (days)			SEM
	20 th day	30 th day	40 th day	
BPS	6.00	6.55	6.90	0.18
EPB	6.16 ^b	6.10 ^b	7.00 ^a	0.17
RHL	6.82 ^b	6.97 ^{ab}	7.00 ^a	0.03

^{abc} means with different superscript in a row are significantly different ($p < 0.05$), BPS = Banana pseudo-stem, EPB = Empty palm bunch, RHL = Rice hull/husk

Although there were no significant changes in the pH of the BPS, a progressive increase could also be observed. [21] reported that pH of 6 is optimal for *P. tuber-regium* growth, indicating that most of the values recorded in the present study are slightly high. The changes in the temperature and pH of the BPS indicate some fermentation activities although the *P. tuber-regium* could not colonize it. Several studies have reported successful ensilage of BPS through the activities of endogenous bacteria and fungi [35, 36] or commercial yeast [37]. It is suggested that endogenous organisms aided the fermentation of the BPS during the 40 days experimental period.

Effects of *P. tuber-regium* fermentation on the physical characteristic of biomass wastes

The bulk density, water holding capacity, oil absorption capacity and swelling capacity of the raw and non-fermented banana pseudo-stem (BPS), empty palm bunch (EPB) and rice hull (RHL) produced at different locations in southeastern Nigeria are shown in tables 4, 5 and 6 respectively. The result of the physical characteristics of BPS as shown in table 4 revealed that the fermentation process significantly ($p < 0.05$) reduced the water holding capacity (WHC), and swelling capacity (SWC) values up to the 40th day of fermentation. The bulk density (BD) and

oil absorption capacity (OAC) were however reduced significantly ($p < 0.05$) up to the 30th day of fermentation. Indeed, the BD which is a measure of the mass of the given volume of a material [38] decreased significantly with increasing fermentation days, suggesting that the BPS undergoes changes in its physical structure during fermentation thus leading to the increase in its BD. [39] reported a similar 0.43g/ml BD value for BPS flour, while [40] and [41] reported lower values (0.385 and 0.21g/ml respectively). The WHC and SWC of a material which refer to the ability of the material to retain water [42, 43] were highest in the control sample, suggesting that BPS tends to lose its water retention ability with increase in fermentation period. According [44] the SWC is directly related to the amount of cellulose in the dietary fiber of material. The WHC results were lower than the 744.02% reported by [41] for partially decomposed BPS. [44] however reported a higher WHC (1540%) and SWC (125.8%) in BPS from India. [39] also reported a higher WHC value (1066.0%). The OAC which represents the amount of oil a material can absorb [45] and is attributed to the chemical and physical structure of the plant polysaccharides [44] was significantly reduced by the fermentation process. This may explain the lower value (365.56%) reported by [41] in partly decomposed BPS than the control value recorded in the present study. [39] and



[44] however reported relatively similar values of 545% and 475% respectively in BPS from Asia. These variations in the physical characteristics results could be attributed to differences in banana varieties, stages of plant development, and processing methods [46, 41]. Similarly, since the *P. tuber-regium* could not utilize the BPS as a fermentation substrate, it could be concluded that the changes recorded in its physical characteristics during the treatment periods were caused by ensiling bacteria and fungal organisms.

The physical characteristics results of empty palm bunch (EPB) as presented in table 5 shows that the control

sample values for all the parameters studied were significantly higher ($p < 0.05$) than the other treatment values, indicating significant effects of the fermentation process on the physical characteristics of the biomass material, especially WHC and OAC values. The 40th day SWC value was however similar to the control value, indicating that the fermentation effects terminated between days 30 and 40. The BD values of the fermented samples were however significantly higher ($p < 0.05$) than that of the control.

Table 4: Effects of *P. tuber-regium* fermentation on the physical characteristics of the banana pseudo-stem

Parameters	Fermentation periods (Days)				SEM
	T1 (0)	T2 (20)	T3 (30)	T4 (40)	
BD (g/mol)	0.473 ^a	0.489 ^a	0.371 ^b	0.393 ^a	0.02
WHC (%)	561.310 ^a	515.513 ^{bc}	518.250 ^b	506.110 ^c	6.56
OAC (%)	384.813 ^a	335.987 ^c	358.237 ^b	395.203 ^a	7.12
SWC (%)	765.35 ^a	702.273 ^c	715.103 ^b	613.077 ^d	16.60

^{abc} means with different superscript in a row are significantly different ($p < 0.05$), BD = Bulk density, WHC = Water holding capacity, OAC = Oil absorption capacity, SWC = Swelling capacity

Table 5: Effects of *P. tuber-regium* fermentation on the physical characteristics of the empty palm bunch

Parameters	Fermentation periods (Days)				SEM
	T1 (0)	T2 (20)	T3 (30)	T4 (40)	
BD (g/mol)	0.251 ^b	0.311 ^a	0.304 ^a	0.300 ^a	0.01
WHC (%)	405.343 ^a	364.007 ^b	305.037 ^d	327.327 ^c	11.58
OAC (%)	454.377 ^a	243.057 ^c	295.057 ^b	239.200 ^c	26.40
SWC (%)	523.907 ^a	464.937 ^c	504.520 ^b	534.343 ^a	8.14

^{abc} means with different superscript in a row are significantly different ($p < 0.05$), BD = Bulk density, WHC = Water holding capacity, OAC = Oil absorption capacity, SWC = Swelling capacity.

The result suggests that the fermentation process resulted in increases in the mass or reductions in the porosity of the EPB biomass waste. The BD values are generally lower than the values reported in other biomass wastes such as BPS [39, 40] and RHL [48], which agrees with the report of [48], reported that one of the physical traits of EPB is low BD. The changes observed in the physical characteristics of the fermented EPB could be attributed to the biophysical effects of the *P. tuber-regium*

growth on the biomass. [13, 14] reported similar effects of *P. ostreatus* and *V. volcacea* on EPB during fermentation and attributed it to the effects of these mushrooms on the different components of the fiber fractions of the biomass during their growth.

Table 6 shows the results of the physical characteristics of rice hull/husk (RHL). The control sample values were generally significantly higher ($p < 0.05$) than the values recorded in the fermented samples. The OAC values on



the 30 day of fermentation was however found to be statistically similar ($p>0.05$) to the control value. The WHC and SWC values reduced to the lowest levels by the 20th day and subsequently increased with increasing fermentation days, although the 40th day value remained significantly lower than the control value. These negative changes in BD and SWC values reflect the probable alterations in the structure and properties of the RHL during fermentation. [49] reported higher values of WHC (235%) and OAC (240%) for non-fermented rice husk,

while [50] reported a lower BD range (331.5 – 380%). These variations in physical characteristics have been attributed to genetic variation in rice grains, and processing methods [51, 52]. [53] reported that the crude fiber in rice straw treated with *P. tuber-regium* reduced significantly indicating that the general reductions in the physical characteristics observed in the present study may be driven by reductions in crude fiber values. [54] also suggested that *P. ostreatus* treatment could enhance the degradation of rice husk.

Table 6: Effects of *P. tuber-regium* fermentation on the physical characteristics of the rice hull/husk

Parameters	Fermentation periods (Days)				SEM
	T1 (0)	T2 (20)	T3 (30)	T4 (40)	
BD (g/mol)	0.472 ^a	0.465 ^{ab}	0.419 ^{bc}	0.399 ^c	0.01
WHC (%)	225.097 ^a	197.070 ^c	204.707 ^b	208.613 ^b	3.14
OAC (%)	225.343 ^a	205.920 ^b	227.117 ^a	214.257 ^b	2.83
SWC (%)	335.293 ^a	304.973 ^c	324.790 ^b	288.847 ^d	5.47

^{abc} means with different superscript in a row are significantly different ($p<0.05$), BD = Bulk density, WHC = Water holding capacity, OAC = Oil absorption capacity, SWC = Swelling capacity.

Conclusions

It was concluded that *P. tuber-regium* could colonize the milled seven days composted empty palm bunch (EPB) and rice hull/husk (RHL) but could not colonize banana pseudo-stem (BPS). The optimal fermentation pH was slightly acidic (6.00 - 6.82) during the early stage of the fermentation but became neutral (6.90 - 7.00) by the 40th day of fermentation. 20 days of *P. tuber-regium* fermentation of the composted EPB and RHL were adjudged optimal based on the improvements in their physical characteristics.

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