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Evaluation of Cashew Pulp and Shell, Kola, Cocoa and Coffee Husk as Substrates for the Cultivation of *Pleurotus ostreatus*

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Authors' contributions

This work was carried out in collaboration between both authors. Author STL designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors EO performed the experiments of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Coffee, cocoa, kola and cashew shells and cashew pulp, all agricultural waste were evaluated as potential raw materials for cultivating *Pleurotus ostreatus* and to determine the influence of substrate on mushroom nutritional quality.
Methodology: Composted substrates were bagged, inoculated with spawn and cropped. Treatments were arranged in a Completely Randomised Design in the cropping house. Substrate colonization, yield and nutritional quality of mushroom carried out using various biochemical tests.
Results: Mushroom grown on cashew pulp had significantly lower levels of total polyphenols than the other five substrates suggesting that bioaccumulation of phenolics by the fungus was not dependent on the levels originally present in the substrate. Carbohydrates, one of the major constituents of mushroom, ranged from 11% in cocoa to 20.6% in cashew pulp. In terms of soluble sugars, cashew pulp mushroom had significantly ($P < .05$) higher levels of soluble sugars than the

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others. Heavy metal levels were all low and within the recommended for food products. Low crude fat content characterized the samples, significantly influenced by substrate and ranged from 0.95% (Kola husk) to 2.83% (cocoa pod husk) in dry mushrooms. Significant differences observed in the dry matter content of the mushroom grown on the six substrates, with values ranging from 8.01 to 13.96%. Mushroom from cashew shell and coffee husk had the lowest dry matter among the treatments.

Conclusion: Kola husk, cocoa pod husk, cashew pulp and cashew shell as available agricultural wastes produced in the country have high potential for utilization as substrates for the cultivation of *P. ostreatus* mushroom with good nutritional value. Some nutrient compositions were however influenced by the substrate used. All substrates supported *P. ostreatus* growth fruiting, however coffee husk did produce lower yields in terms of biological efficiency.

Keywords: Cashew; cocoa; coffee; kola; agricultural waste; Pleurotus ostreatus.

1. INTRODUCTION

Ghana produces coffee, cocoa, cashew, shea and kola as agricultural crops for export and local consumption. A lot of agricultural waste is however generated in the wake of this production.

The cashew fruit has various important uses. The pulp juice and the nuts are usually processed for human consumption but the pulp is usually left to go waste with limited processing for animal feed.

In the case of kola, the nut is also processed and put to various uses. The kola husk has been found to be useful in certain areas. In Nigeria, kola nut testa (husk) has been suggested as a possible ingredient for making fertilizers due to the high potassium content [1-3]. The kola pod husk has also been utilized for the production of liquid soap. The most recent and remarkable advancement in kola by-product utilization is the use of kola pod husk in the replacement of up to 60% of the maize used in poultry feed formulations [3-10]. In Ghana, the shea fruit pulp is partly utilized as food by the natives, coffee husk as mulch and cashew pulp as animal feed. The cocoa pod husk is also utilized in soap making, animal feed production and fertilizer and recently as substrates for mushroom cultivation [11-13]. With all these known uses of the kola and coffee husk, shea pulp and cashew pulp it is expedient to research into other important uses of these materials, which are mostly discarded as waste in Ghana.

Most agricultural wastes such as cashew pulp, coffee and kola husk are known to contain lignin and cellulose. Oyster mushroom is a macroscopic fungus and an example of liginocellulolytic mushroom, a class of mushrooms that grows on both cellulose and lignin containing substrates [14].

In mushroom cultivation, sawdust has been used as a substrate [15]. The idea of mushroom cultivation has now been shifted onto agricultural and industrial waste [16-19]. Mushrooms are considered as rich food sources because they contain among other things protein, glycogen, sugars, lipids, vitamins, crude fibres and amino acids. They also contain minerals required for the normal functioning of the body [20-22]. Factors such as growing site, type of substrate, mushroom type, and developmental stages and parts of the fungal samples analyzed however influence the nutritional composition [23,24].

Oyster mushrooms, *Pleurotus spp*. are widely cultivated on a variety of agricultural wastes. This makes it easy and least expensive to grow and they perform appreciably well in tropical and subtropical climates. Their cultivation on a variety of agricultural wastes has the advantage of putting these wastes to some use and thus alleviating the incidence of environmental pollution [25]. The degraded materials after the cultivation of the mushroom also have the added advantage of being processed into animal feed after the lignocellulose's breakdown. The aim of this work was to establish standard procedures for cultivating edible mushrooms from cashew, cocoa, shea, Kola and coffee waste and determine the influence of substrate on nutritional quality.

2. MATERIALS AND METHODS

Mycelial growth rate on the different substrates, Total yield, Biological efficiency and dry weight of the mushroom were calculated.

2.1 Composting and Bagging

Sixty kg of Sawdust of "wawa" (*Triplochiton scleroxylon*), cashew pulp, kola, cocoa and coffee husk were each heaped and composted over a 28 day period whilst the cashew shells were heaped for 120 days. Adequate water (4.5L) was added and turning done in an interval of four days to enhance microbial growth.

Determined (Kjeldahl method [26]) percentage nitrogen contents of sawdust, cocoa pod husk, cashew pulp, Kola pod husk, coffee husk and cashew shell used in the work were respectively 0.21%, 2.75%, 2.40%, 1.85%, 1.47% and 0.88%.

Using sawdust as a control, kola, cashew and shea pulp and coffee and cocoa husk were either bagged unamended or wheat bran (10%) and lime (1%) added as additives. These combinations were put in heat resistant polypropylene bags (33 cm x 10.5 cm). The bags were steam sterilized by heating in closed metallic drums for three hours. The bags were then allowed to cool at room temperature for 24 hours.

2.1.1 inoculation, incubation and cropping

About 10-15 seeds of grains were transferred into each bag and bags plugged with non-absorbent cotton wool and the mycelium allowed to run through the bag. Reading of the rate of growth of mycelium in the bag was recorded with a ruler. After total colonization, the bags were sent to the cropping house. A temperature between 21 – 27°C and relative humidity between 75 – 95% was maintained at the cropping house. The colonized compost bags were allowed to acclimatize in the cropping house by sprinkling controlled amount of water for 3 to 4 days on the bags. The bags were then sliced opened at one end and fruiting bodies allowed to emerge from the bags.

2.2 Biochemical Analysis

2.2.1 Moisture content

The fresh weight of each mushroom sample was taken using a chemical balance. These samples were then dried in an oven at 80°C for 48 h. The loss in weight was regarded as the moisture content [27].

2.2.2 Ethanol soluble sugars

An amount of 0.5 g powdered mushroom sample was extracted with 30 ml of 80% ethanol for 6 h and the amount of sugar in the extract determined using phenol sulphuric acid method [28]. For individual sugar determination and identification, 20 ml of the resulting extract was rotary evaporated and residue dissolved in 5 ml HPLC grade water. Xylose (50 mg) was added as an internal standard. Prior to injection of 20 µl sample was filtered through 0.45µm filter.

Analyses for soluble sugars (glucose, mannitol, trehalose, and mannose) were carried out on a Waters liquid chromatography system comprising of a waters 1525 Binary pump with 2414 Refractive index detector. An ionic exchange column, Aminex HPX-87H (300 x 7.8mm, Bio-Rad Laboratories Ltd) coupled to a pre-column of cationic exchange (Bio-Rad Laboratories Ltd) was used to achieve chromatographic separation. Mobile phase was a 0.005 mol L^{-1} H_2SO_4 solution with flow-rate of 0.6 ml min^{-1}. Sugars were identified and their concentrations determined by comparison with retention times of authentic standards and internal standard.

2.2.3 Ash content

An amount of 3 g powdered mushroom was ashed in a furnace in a previously ignited and cooled crucible of known weight at 550°C for 6 h. The cooled crucibles were put in desiccators and weighed [29]. The ash content was then calculated.

2.2.4 Lipid and total nitrogen content

An amount of 2g powdered sample was extracted with 30 ml of petroleum ether in a soxhlet extractor for 4 h. After evaporation of solvent in a rotary vacuum evaporator, the weighed flask was dried in an oven at 80°C for 2 h, allowed to cool and reweighed. The difference between the two weighing was computed as the lipid content [30]. Nitrogen was determined using AOAC official method 977.02 [26]

2.2.5 Mineral nutrients

Chromium, Cadmium, Cobalt, Manganese, Iron, copper, Nickel, and Zinc was determined using Atomic absorption spectrometry (AAS) [31] after microwave digestion of the material.

3. RESULTS AND DISCUSSION

3.1 Substrate Fermentation

Mushroom mycelia growth is dependent on the nutrients available for their growth obtained from the substrate. Composting of agricultural waste is therefore an important and a first step in the mushroom production process. This process sometimes critically influences the pH (acidity) of the material that will later influence the decision to lime the substrate to a certain pH as well as to buffer the substrate pH to suit the particular mushroom to be cultivated. The initial pH range for all the substrates was between 4.77 – 6.11 with Coffee husk and Cashew shells being the most acidic at the beginning of the fermentation. As fermentation progressed the pH of the substrates increased significantly for all the substrates except cashew shells. The pH of the coffee husk increased gradually during the composting to about 7 while that for the cashew shell virtually did not change over the six week period. Cocoa pod husk had the highest change in pH, increasing from 5.2 to 9.5 (Fig. 1).

The *Pleurotus ostreatus* mushroom colonized the substrate much easily when grown on sawdust and least growth was observed on cashew shell (Fig. 2). The cashew pulp was however observed to be highly prone to contamination among all the substrates. Growth rate of the fungal mycelia on cocoa and kola pod husks were not different from each other.

Mushrooms have been reported to be important sources of phosphorus, sodium, potassium and vitamin C [14]. Table 1 indicates some selected elemental composition of the mushrooms grown on various substrates. Levels of heavy metals like Cadmium (Cd), Cobalt (Co) and Chromium (Cr) were very low and within recommended levels for food. Iron and copper were however relatively high in mushrooms grown on Kola and Cocoa pod husk substrates. The values for iron (Fe) however obtained in this study are about ten times less what has been reported in *Pleurotus* spp from sawdust substrate in Ghana [32]. This difference could be attributed to the difference in the substrates used. Dietary copper intake has been reported to vary with the types of food consumed, the condition of the soils (e.g., copper content, pH, etc.) from which foods are produced and drinking-water characteristics. The relatively high levels of copper in the cocoa and kola husk grown mushroom could be attributed more to the soil from which they were grown. Even though copper based fungicides are used on cocoa to control Phytophthora, they may have contributed very little to the levels as copper based fungicides are not applied to kola but kola substrate grown mushroom tended to have high levels as well.

Copper plays an essential role in maintaining normal health in animals and humans. The average daily dietary requirement for copper in the adult human has been estimated at 2 mg and for infants and children at 0.05 mg/kg bw [33,34]. The National Research Council [35] reported "estimated safe and adequate" daily dietary intakes of copper ranging from 0.5 to 0.7 mg/day for infants 6 months of age or less up to 2-3 mg/day for adults. The amounts reported here are safe and would form a significant source of copper when these substrates are used for mushroom cultivation.

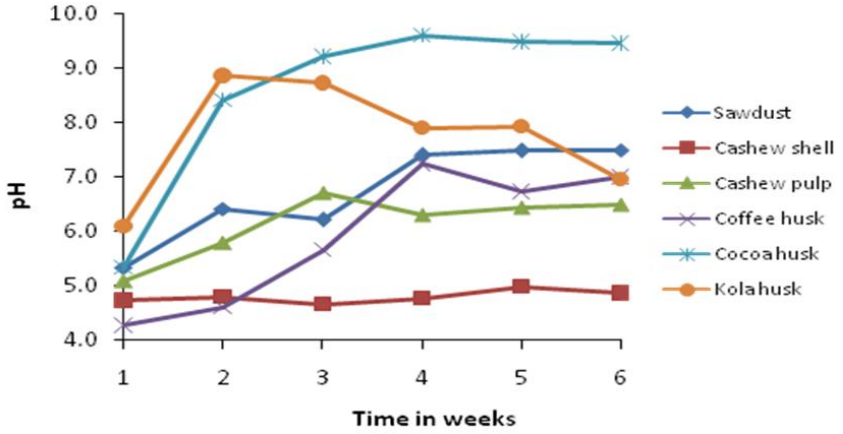

Fig. 1. pH of cocoa, coffee, cashew and sawdust substrates during composting over a period of 6 weeks

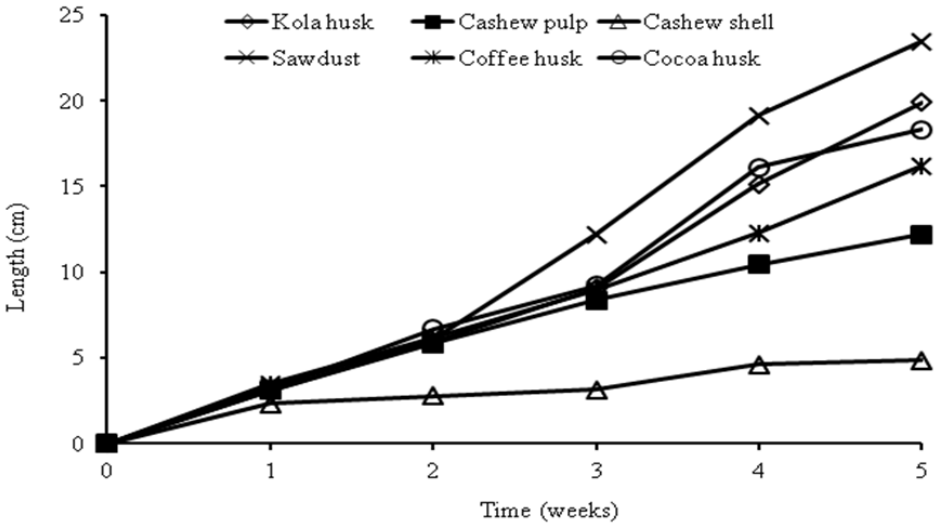

Fig. 2. Graph showing growth rate of *P. ostreatus* on the six substrates

Table 1. Mineral composition of mushroom harvested from various unamended substrates

Substrate	Concentration (mg/Kg)							
	Fe	Mn	Cu	Zn	Cr	Ni	Cd	Co
Cocoa pod husk	51.77^{a}	4.55^{a}	25.57^{a}	15.94^{a}	<0.006^{a}	1.71^{a}	<0.002^{a}	<0.005^{a}
Cashew shell	25.73^{b}	1.30^{b}	16.18^{b}	8.84^{b}	<0.006^{a}	0.08^{b}	<0.002^{a}	<0.005^{a}
Cashew pulp	25.73^{b}	1.30^{b}	16.18^{b}	8.84^{b}	<0.006^{a}	0.54^{c}	<0.002^{a}	<0.005^{a}
Saw dust	30.41^{c}	0.97^{c}	15.82^{b}	12.99^{c}	<0.006^{a}	0.29^{d}	<0.002^{a}	<0.005^{a}
Kola husk	52.55^{d}	3.44^{d}	23.79^{a}	7.16^{d}	<0.006^{a}	1.88^{a}	<0.002^{a}	<0.005^{a}
Coffee husk	49.18^{e}	3.85^{d}	14.34^{b}	15.10^{a}	<0.006^{a}	<0.001^{e}	<0.002^{a}	<0.005^{a}

Means with different letters within a column are significantly different (P < 0.05)

Mushroom grown on cashew pulp had significantly lower levels of total polyphenols than the other five substrates (Table 2). This was found to be surprising because the level of polyphenols in the cashew pulp and shell substrates were found to be much higher than in the other substrates. The results tend to suggest that bioaccumulation of phenolics by the fungus was not dependent on the levels originally present in the substrate. Though levels of o-dihydric phenols were generally low, cashew pulp had significantly ($P < 0.05$) higher amounts compared to the other five substrates. Carbohydrates have been reported to form one of the major constituents of mushroom, with ranges from 27.1% to 81.8% [23,32,36,37,38]. In this study the total carbohydrate content of the mushrooms were much lower than has been reported in literature, ranging from 11% (cocoa pod husk) to 20.6% in cashew pulp grown mushroom (Table 2). In terms of soluble sugars, cashew pulp mushroom had significantly ($P < 0.05$) higher levels of soluble sugars than the others. This could be attributed to the fact that the cashew pulp substrate contained relatively higher residues of sugars after the juice extraction and therefore directly bio-accumulated by the mushroom.

The soluble sugar components were mainly four with glucose and mannose being dominant. Glucose was found in the highest amounts across the substrates (Table 3). Mannitol and trehalose, the main representatives of alcoholic sugars and oligosaccharides, respectively [37,39, 40] were present with trehalose being the least across the substrates. These soluble sugars reportedly control and contribute a sweet or sugary taste to this class of mushroom.

The low crude fat content (extracted as free fatty acids, tri-, di- and monoglycerides, phospholipids, sterols, and derivatives [41]) of mushrooms generally makes them acceptable low-calorie diet for consumption [42]. Generally all the substrate grown mushroom had low levels

crude fat. The fat contents however varied depending on the substrate used (Table 3). Cocoa pod husk substrate significantly produced mushrooms with relatively higher fat content compared to the others. The fat content widely ranged from 0.95% (Kola husk) to 2.83% (cocoa pod husk) in dry mushrooms. The fat yield obtained from the mushrooms grown on the cashew shell and saw dust were not significantly different ($P > 0.05$). Mushroom from kola and coffee husk also contained the same amount of crude fat. The range of crude fat yields obtained in this study falls within reported values for *Pleurotus* spp [32,42] as well as for other species cultivated on various substrates. The percentage ash content of mushrooms has been reported to be low and between 5 and 12% of dry weight [38,43]. Values obtained from the mushrooms emanating from the various substrates ranged from 6.2% in cashew pulp to 9.0% in kola husk. Ash content of mushroom grown on cashew shell and sawdust did not significantly differ from each other. Dry weight content of mushrooms is an important factor considered when looking at their nutritional value and this is reported to range between 5–15% for various mushrooms [26,42,44]. Current study showed significant differences in the dry matter content of the mushroom grown on the six substrates, with values ranging from 8.01 to 13.96%. Mushroom from cashew shell and coffee husk had the lowest dry matter among the treatments.

3.2 Moisture Content

Fresh *Pleurotus* mushroom is generally reported to contain 85-95% moisture [45] and dependent on the environmental factors (temperature and humidity) during growth and storage, averaging around 90% [37,41,46]. The moisture content of the harvested mushroom fruit bodies were found to range from 89% to 92% (Table 4). Substrate did not seem to influence the level of moisture in the fruit bodies. This results agrees with previous ones obtained from other substrates reported [32].

3.3 Crude Protein

Proteins form one of the main constituents of importance in mushrooms. Crude protein levels ranged from 20.41 to 25.90 in the samples of mushroom (Table 4). Available literature indicates that protein content on a dry weight basis in mushrooms in general range between 10 and 40%, and varied extensively among and within the species [47]. For *Pleurotus,* a range between 20 and 25% is typical [47]. Factors affecting the protein content in general include, the type of mushroom, the stage of maturation, the part of mushroom body and availability of nitrogen content in the substrate [48]. The protein levels in mushroom obtained from the various substrates fell well within the reported literature with cocoa pod husks as substrate yielding mushrooms with significantly higher protein.

3.4 Biological Efficiency

The biological efficiency based on the dry weight of each substrate is presented in Table 5. Maximum bioefficiency of *Pleurotus ostreatus* was on sawdust and the least on coffee husk. Coffee husk has however been reported to be very suitable for mushroom cultivation when it is amended with other substrates before the cultivation of the mushroom. Results obtained in study suggest that amending all the substrates used in this study will give a high turnover of mushroom than when no additives are added.

Table 2: Phenolic and carbohydrate composition of mushroom grown on the six unamended substrates

Substrate	Phenolics (mg/g)		Carbohydrates (mg/g)	
	O-dihydric	Total	Soluble	Insoluble
Cocoa pod husk	0.22 ± 0.03^{a}	5.09 ± 0.38^{a}	80.73 ± 1.4^{a}	29.55 ± 0.3^{a}
Cashew shell	0.25 ± 0.04^{a}	6.11 ± 0.37^{b}	88.14 ± 2.0^{b}	34.16 ± 1.6^{b}
Cashew pulp	0.37 ± 0.01^{b}	4.46 ± 0.17^{c}	158.67 ± 0.5^{c}	47.82 ± 2.3^{c}
Coffee husk	0.22 ± 0.05^{a}	5.58 ± 0.18^{b}	111.89 ± 3.0^{d}	43.67 ± 2.7^{c}
Sawdust	0.20 ± 0.04^{a}	5.92 ± 0.06^{b}	134.89 ± 8.1^{e}	27.78 ± 1.6^{a}
Kola husk	0.22 ± 0.04^{a}	5.57 ± 0.22^{b}	154.25 ± 3.4^{f}	32.03 ± 0.4^{f}

Each value is expressed as mean ± SD (n = 5). Means with different letters within a column are significantly different (P < 0.05)

Table 3. Soluble sugar composition (mg/g) of mushroom grown on the six unamended substrates

Substrate	Glucose	Trehalose	Mannitol	Mannose
Cocoa pod husk	32.10 ± 0.35[a]	3.83 ± 0.20[a]	21.00 ± 0.44[a]	23.80 ± 0.15[a]
Cashew shell	35.05 ± 0.22[b]	4.18 ± 0.67[a]	22.93 ± 0.69[b]	25.99 ± 0.27[a]
Cashew pulp	63.09 ± 0.77[c]	7.52 ± 0.99[b]	41.28 ± 0.10[c]	46.78 ± 0.44[c]
Coffee husk	44.49 ± 0.75[d]	5.31 ± 0.35[c]	29.11 ± 0.13[d]	32.99 ± 0.36[d]
Sawdust	53.63 ± 0.23[e]	6.40 ± 0.89[cd]	35.09 ± 0.42[e]	39.77 ± 0.29[e]
Kola husk	61.33 ± 0.21[f]	7.31 ± 0.15[d]	40.13 ± 0.04[f]	45.48 ± 0.42[f]

Each value is expressed as mean ± SD (n = 3). Means with different letters within a column are significantly different (P < 0.05)

Table 4. Percentage Ash, fat and moisture content of mushroom grown on the six substrates

	Percentage (%)				
Substrate	**Ash**	**Fat**	**Moisture**	**Dry matter**	**Crude protein**
Cocoa pod husk	7.2 ± 0.4[a]	2.83 ± 0. 16[a]	90.7 ± 4[a]	13.96 ± 0.22[a]	25.90 ± 0.78[a]
Cashew shell	8.1 ± 0.2[b]	1.51 ± 0.03[b]	89.1 ± 2[a]	9.01 ± 0.72[d]	20.41 ± 0.72[c]
Cashew pulp	6.2 ± 0.6[c]	1.32 ± 0.02[d]	91.6 ± 5[a]	10.91 ± 1.04[c]	23.79 ± 1.03[b]
Coffee husk	7.7 ± 0.3[a]	0.99 ± 0.01[c]	89.8 ± 3[a]	8.01 ± 0.52[d]	21.44 ± 0.52[d]
Saw dust	7.6 ± 0.2[a]	1.57 ± 0.05[b]	92.1 ± 8[a]	12.04 ± 0.25[b]	22.64 ± 0.25[b]
Kola husk	9.0 ± 0.5[b]	0.95 ± 0.08[c]	90.2 ± 3[a]	13.25 ± 0.62[a]	24.89 ± 0.92[a]

Each value is expressed as mean ± SD (n = 3). Means with different letters within a column are significantly different (P < 0.05)

Table 5. Biological efficiency, weight and average yield of mushrooms from the substrates

Substrate	Weight of substrate (g)	Average yield of flushes (g)	Biological efficiency (%)
Cocoa pod husk	1000	610.0 ± (5.0)	61.0
Cashew shell	1000	440.4 ± (5.5)	44.0
Cashew pulp	1000	415.9 ± (7.3)	41.6
Coffee husk	1000	200.6 ± (2.8)	20.6
Sawdust	1000	640.0 ± (3.7)	64.0
Kola husk	1000	615.0 ± (4.1)	61.5
*Cocoa pod husk	1000	724.0 ± (5.6)	72.4
*Cashew shell	1000	550.0 ± (3.3)	55.0
*Cashew pulp	1000	522.0 ± (2.9)	52.2
*Coffee husk	1000	568.0 ± (4.8)	56.8
*Sawdust	1000	700.4 ± (3.8)	70.0
*Kola husk	1000	707.0 ± (12.3)	70.7

** Substrates amended with wheat bran (10%) and lime (1%) as additional nitrogen source and pH value control in the substrates respectively. Figures in brackets represent standard errors.*

4. CONCLUSION

Kola husk, cocoa pod husk, cashew pulp and cashew shell as available agricultural wastes produced in the country have high potential for utilization as substrates for the cultivation of *P. ostreatus* mushroom. Substrate did not affect fruit body percent moisture content. Some nutrient compositions were however influenced by the substrate used. All substrates supported growth and fruiting of *P. ostreatus*. However coffee husk did produce lower yields in terms of biological efficiency. The nature of the cashew shells also made it imperative to have a longer composting period before being suitable for use. Amending the substrates with wheat bran increased the biological efficiency.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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