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Effect of substrate media on growth, yield and nutritional composition of domestically grown oyster mushroom (*Pleurotus ostreatus*)

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The effect of substrate (medium) on growth, yield and nutritional composition of domestically-grown oyster mushroom (*Pleurotus ostreatus*) was investigated. Six different substrates namely sawdust only (SDO), sawdust + corn waste + $CaCO_3$ (SDW), sawdust + rice bran + $CaCO_3$ (SDR), sawdust + banana leaves (SBL), sawdust + cassava peel (SDC) and cassava peel only (CPO) were used. The substrates were pasteurized with hot water (90°C for 4 h) before spawns of oyster mushroom were inoculated to them. After inoculation, the substrates were kept in a controlled environment until fruiting took place. The SDC substrate gave the highest number (22) of fruiting body, highest yield (463 g/kg) and best biological efficiency (46.30%). This was followed closely by the harvest from SDR substrate. The differences in the nutrient composition of mushroom from the different substrates were significant at 0.05 % confidence level. Harvest from SDR contained higher vitamins and minerals compared to others. Harvest from CPO substrate had the lowest (20.10%) protein content as well as other nutrients. SDC and SDR substrates are considered good for domestic cultivation of oyster mushroom.

Key words: Mushroom, substrate, efficiency, home-grown, nutrients.

INTRODUCTION

Widespread malnutrition with ever increasing protein gap in the third world including Nigeria has necessitated the search for alternative protein. Mushroom is among the favoured alternatives. Mushrooms belong to the kingdom of fungi, a group, very distinct from plants, animals and bacteria. They lack the most important features of plants - the ability to use energy from the sun directly through chlorophyll. Thus, fungi depend on other organisms for food, absorbing food from the organic materials in which they live (Oei, 2005; Ha et al., 2015). Hence mushrooms exist as saprophytes on trees, and this is why forests are often generous to mushroom hunters. The oyster mushroom is a primary decomposer of wood. However, mushroom should be harvested from hardwood only, as those growing on soft wood are poisonous (SOMA, 2017; Zhao, 2009). Mango, avocado pear and African bread fruit trees are among the commonest trees on which mushrooms and in particular the local *Pleurotus tuber-*

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Table 1. Substrate formulation for cultivation of oyster mushroom.

Substrate code	Composition
SDO	Sawdust only (100%)
SDR	Sawdust (78%) + Rice bran (21%) + $CaCO_3$ (1%)
SDW	Sawdust (89%) + Corn waste (10%) + $CaCO_3$ (1%)
SDC	Sawdust (75%) + Cassava peel (25%)
SBL	Sawdust (75%) + Banana leaf (25%)
CPO	Cassava peel only (100%)

regium (*atakata elo*) grow naturally (Chinda and Chinda, 2007). In the remote virgin forest areas, decaying remains of trees crystallize into what is generally referred to as "usu", a white tissue of mycelium colony from which the flowering part (mushroom) develops naturally in the face of favourable conditions and humidity. While most farmers regard "usu" as a food condiment, some more knowledgeable ones have learnt to bury (cultivate) this substance in their farms, from which they harvest mushrooms at specific period in the year, usually during rainy season when the conditions are favourable. Since mushrooms are seasonal, commercial cultivation is therefore necessary to ensure constant availability. However, large scale cultivation and processing of mushroom requires a good knowledge of the growth requirements, and influence of the substrate on their growth rate and nutritional composition. Some researchers have already observed that the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates (Badu et al., 2011; Tesfaw et al., 2015). Hence the objective of this study is to determine the effect of different mixture of substrate media on growth characteristics and nutritional composition of domestically grown oyster mushroom.

MATERIALS AND METHODS

The substrates namely: Rice bran, Corn waste, Banana leaves, Sawdust and Cassava peel were sourced locally from Njaba LGA of Imo State, Nigeria while the spawn was obtained from Dilomat Mushroom Farms and Research Centre, River State University of Science and Technology (RSUST), Nigeria.

Preparation and formulation of substrates

The banana leaves and cassava peels were dried and milled separately into powdered form. The individual substrate combinations were well mixed (the formulation of the substrates is shown in Table 1) and soaked in water for 24 h to moisten them. Subsequently they were stalked on steep cemented floor so as to remove excess moisture from the substrates to get 65% moisture level. The entire substrates were fermented for 3 days by covering them with polythene sheets before bagging. After fermentation each substrate was filled into heat resistant plastic bag (100 gauze thickness), measuring 15 cm, and compressed to make bag logs weighing 1.0 kg (Plate 1). The openings of the bags were closed with a plastic ring and cotton wool plug. The substrates were then pasteurized by partly immersing them in hot water (90°C for 4 h).

Plate 1. Heat resistant plastic bags filled with substrates for mushroom cultivation.

After heat treatment, the bags were cooled to 30°C before inoculating with the spawn of oyster mushroom (*Pleurotus ostreatus*) at the rate of 20 g per 1.0 kg bag of substrate. The substrates (now bagged and inoculated) were incubated in a darkroom for 3 to 4 weeks on a shelf. During this period, daily temperature and humidity of the incubation room were taken twice daily. The bags were fully colonized by the mushroom mycelia within 17 to 30 days. Next the bags were moved to another room for fructification. The two ends of the bags were cut open with a blade and placed side by side on the shelf provided for this purpose. The humidity of the bags during the cropping (fructification) stage was accomplished by spraying of water in the form of fine mist from a nozzle three times a day. Temperature and humidity of the cropping room were also monitored two times a day. Exhaust fans were used for exhaust of gases from cropping room to ensure adequate oxygen supply to the spawns. The first primordial (pin heads) appeared 7 to 10 days after opening the bags depending upon the substrate. Matured mushroom were harvested by twisting gently to uproot from the base. The mushrooms generally mature in two to three days after the appearance of the pin heads.

Analysis of growth rate of oyster mushroom

The yield of oyster mushroom was determined by recording the number and size of cap of the fruit bodies after sprouting. The following parameters of growth and yield were measured.

Mycelium running time

This is the number of days it took the mycelium to fully colonize the substrate bags.

Number of fruit bodies

This was done by directly counting the number of fruit bodies on each bag/ substrate.

Cap size (Pileus diameter)

This was achieved by measuring the broadness of the cap after

harvesting. This was carried out in the morning hours using a measuring ruler.

Cropping time

This is the time from the completion of mycelium running to the time when the pin heads have fully blossomed and ready for harvesting. It was measured in days.

Yield of mushroom

This is the quantity (weight) of mushroom produced per bag of substrate per harvest time. It was weighed with kitchen scale. The crop of oyster mushroom was harvested in four flushes.

Biological efficiency

The biological yield (g/kg) was determined by weighing the whole cluster of the fruiting body divided by the initial weight of the substrate. The biological efficiency was calculated thus:

$$\text{Biological efficiency (\%)} = \frac{\text{Total yield (kg)}}{\text{Weight of substrate used (kg)}} X \frac{100}{1}$$

Analysis of samples

The proximate analysis was conducted in accordance with standard methods of AOAC (2000). Parameters evaluated included moisture, ash, crude fat, crude protein, and dietary fiber. The mineral and vitamin content of the samples were determined using atomic absorption spectrophotometer (Buck Scientific Model 200A System) analysis and chromatographic Shimadzu Cooperation Japan, C-R6A) assay respectively.

Determination of mineral content

The mineral contents were determined using atomic absorption spectrophotometer (AOAC, 2000). First 0.48 to 0.52 g of each sample was weighed into a clean crucible. The crucible was placed in a cool muffle furnace and the temperature of the furnace rose to 500°C for a period of 2 h and still allowed to remain at 500°C for an additional 2 h, then allowed to ash in the oven over night. The ashed sample was removed from the oven and poured into already labeled 50 ml centrifuge tubes. Five mililitres of distilled water was used to rinse the crucibles into the centrifuge tube. The crucibles were further rinsed with 5 ml aqua regia. The process of rinsing with 5 ml aqua regia was repeated two more times to make a total volume of 20 ml, and the sample vortexed for proper mixing. Finally the sample was centrifuged for 10 min at 300 rpm and decanted into clean vials for micro nutrients determination using atomic absorption spectrophotometer.

Preparation of aqua regia solution

Distilled water (1.2L) was poured into a 2L volumetric flask, 400 ml of conc. HCl and 133 ml of 70% Nitric acid added to it and diluted with distilled water to 2 L.

Determination of vitamin B and C

This was done through chromatographic assay (AOAC, 2000). The sample was first homogenized using a mixer blender, and then 2.5 g was put into a 10 ml volumetric flask. Five milliliters of the buffer was added to the flask and shaken with a mechanical shaker for 3 min. More buffers were added to make up the mark of the 10 ml volumetric flask. The solution was filtered and injected into the HPLC. The calibration curve was plotted using calibrant vs absorbance.

$$\text{Concentration (mg/L)} = \frac{\text{Mm/ml (from calibration curve) x 100 x dilution factor}}{\text{Sample weight}}$$

Statistical analysis

Data obtained were subjected to statistical analysis using ANOVA on SASS (9.2) analytical tool on windows 2007. Means were separated using the least significant difference (LSD) at 95% confidence level.

RESULTS AND DISCUSSION

Effect of substrate media on yield of oyster mushroom

The effect of substrate type on yield of oyster mushroom is shown in Figure 1. Generally the substrates had varying ($p<0.05$) effects on the yield of oyster mushroom. The maximum yield (462.0 g/kg) was obtained from SDC substrate followed closely by SDR substrate (396.99 g/kg), while lowest (200.0 g/kg) yield was obtained from SDW substrate. This result is in accordance with the view of other researchers (Daniel, 1985; Narayanasamy et al., 2009; Ahmed et al., 2009) who explained that different substrates give varying mushroom yield because of the biological and chemical differences in the substrates. According to Narayanasamy et al. (2009); Rizki and Tamai, 2011; Anyakorah et al., 2004; Abdurrahman et al. (2009) and Ahmed et al. (2009) suitable nitrogen ratio helps to produce an optimum mushroom yield. Oei (2003) in his work supplemented sawdust with wheat bran (5-10%) in order to enrich his medium for optimum mushroom yield.

Effect of substrate media on growth of oyster mushroom

The substrates media were found to influence duration of mycelium running, pin head formation, number of fruit bodies produced, the cropping time, the pileus diameter (size of cap) and the biological efficiency of oyster mushroom (Tables 2 and 3). The mycelium running took 2 to 3 weeks after inoculation (Table 2). This result agrees with the findings of Oei (2005) and Chinda and Chinda (2007) who reported that spawn running takes 2 to 3 weeks. On the sixteenth day of inoculation, whitish mycelia colonised all the substrates. The CPO and SDC substrates took extra 5 and 7 days respectively before mycelium fully colonized their bags but the mushrooms obtained from these two substrates were larger in size. The broadest size of cap (17 cm) was obtained on SDC and SBL substrates, while the lowest cap size of 10 cm

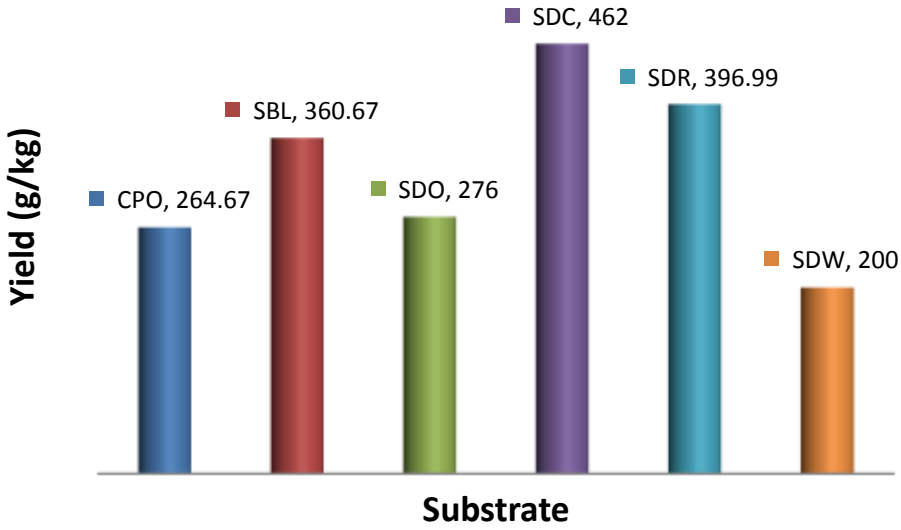

Figure 1. Effect of substrate type on yield of oyster mushroom. SDO = Sawdust only, SDR = Sawdust + Rice bran + $CaCO_3$, SDW = Sawdust + Corn waste + $CaCO_{3,}$, SBL = Sawdust + Banana leaves, SDC = Sawdust + Cassava peel + $CaCO_{3,}$, CPO = Cassava peel only. Data are average of three readings.

Table 2. Effect of substrate media on mycelium running, pin head formation and cropping period of oyster mushroom.

Substrate	Parameters In days			
	Mycelium running	Pin head formation	Pin head to harvest	Cropping duration
CPO	20±0.70	8±0.10	3±0.15	31±0.00
SBL	12±0.10	6±0.42	4±0.16	22±0.00
SDO	15±0.42	7±0.14	3±0.28	25±0.00
SDC	22±0.12	10±0.82	4±0.12	36±0.50
SDR	14±0.35	6±0.14	6±0.20	23±0.00
SDW	14±0.70	6±0.00	3±0.12	23±0.00
Mean	***16±3.92***	***7±1.6***	***4±1.16***	***27±5.6***

SDO = Sawdust only, SDR = Sawdust + Rice bran + $CaCO_{3,}$, SDW = Sawdust + Corn waste + $CaCO_{3,}$, SBL = Sawdust + Banana leaves, SDC = Sawdust + Cassava peel + $CaCO_{3,}$, CPO = Cassava peel only Values are means of duplicatess + standard deviation.

Table 3. Effect of substrate media on biological efficiency, number of fruit bodies and cap size of oyster mushroom*.

Substrate	Weight (g/kg) and number of fruit bodies per flush				Total fruit bodies	Biological efficency (%)	Cap size (cm)
	1st flush	2nd flush	3rd flush	4th flush			
CPO	120(5)	80	40	25	14	26.51	10
SBL	175(8)	100	50	35	20	36.00	17
SDO	150(6)	60	50	15	17	27.50	13
SDC	210(9)	100	98	55	22	46.31	17
SDR	130(8)	110	105	50	20	39.55	15
SDW	100(5)	50	40	10	15	20.00	10
Mean	148(6)±39.96	**83**±24.22	**64**±2.96	**32**±18.35	**332**±3.16	**32.65**±3.12	

*No of fruit bodies per flush is in parenthesis. SDO = Sawdust only, SDR = Sawdust + Rice bran + $CaCO_{3,}$, SDW = Sawdust + Corn waste + $CaCO_{3,}$, SBL = Sawdust + Banana leaves, SDC = Sawdust + Cassava peel + $CaCO_{3,}$, CPO = Cassava peel only Data is a repeart of two croppings.

was obtained on both SDW and CPO (Table 3).

According to Daniel (1985) larger mushroom are produced with longer spawn runs. SDL substrate showed excellent mycelial growth as the bags were fully

Plate 2. Oyster mushroom growing in a domestically prepared media.

colonised in 12 days. The presence of the right proportion of alpha-cellulose, hemi-cellulose, pectin, lignin as well as suitable carbon to nitrogen ratio (Ahmed et al., 2009) might be responsible for the higher rate of mycelium running in SDC and SBL. The ability of mushroom to grow on lingo-cellulosic substrate is related to the vigor of its mycelium (Ashrafuzzaman et al., 2009). A picture of mushroom growing in a domestically prepared media is shown on Plate 2.

The pin-head (the second stage of mycelial growth during cultivation of mushroom) were observed 6 to 10 days after the bags were opened (Table 2). By the 6^{th} day of opening the bags, SBL, SDW and SDR substrates had formation of primordial (pin heads) on them. Sawdust substrate (SDO) brought forth primodia on the 7^{th} day, CPO and SDC substrates brought forth primordia on the 8^{th} and 10^{th} day respectively. These results are in agreement with Oei (2003) and Chinda and Chinda (2007) who observed that oyster mushroom normally complete spawn running in 14 to 28 days depending on the substrates. SDC substrate produced the highest number (22) of fruit body, followed by SBL and SDR (20 each). The fastest cropping time of 36 days was recorded on SDC substrate while the lowest (22 days) was on SBL substrate (Table 3). It is evidence from the data of this research that cassava peels promoted longer spawn runs, broader cap size, as well as longer cropping period. Mushroom samples harvested from SDC, showed the best biological efficiency (46.31%), followed by those from SDR (39.55%), while lowest biological efficiency (20.00%). was recorded on SDW (Table 3).

Effect of substrate media on proximate composition of domestically grown oyster mushroom

There was significant difference ($p<0.05$) among the six substrates in terms of moisture content of mushroom harvested from them (Table 4). Mushroom grown on SDO substrate had the highest moisture content (89.38%), while that grown on SBL substrate had the lowest (72.23%). This findings agrees with the report of Khare et al. (2006), who stated that oyster mushroom grown on banana leaves, usually have low moisture content. For ash content, mushroom harvested from SDW and SDO substrates had the highest ash content of 1.27% each, while those harvested from SDR substrate had the lowest ($p<0.05$) ash content of 0.25%wwb, followed closely by that from SBL with a value of 0.44%. Data in this work differed from those of Behnam and Naser (2008); 5.58% and 6.13% of ash in dried oyster mushroom cultivated in banana straw and rice bran respectively. The highest dietary fibre content of 3.95%wwb was recorded on SDC grown mushroom followed closely by those grown on SBL substrate (3.18%wwb). The lowest dietary fibre content (1.69%) was observed in samples grown on CPO substrate. This means that the dietary fibre content of oyster mushroom (*Pleurotus ostreatus)* was affected by the type of substrate used for their cultivation. The high dietary fibre content of SDC and SBL grown mushrooms might have resulted from the high lignin and cellulose content of cassava and banana leaves, in addition to that already contained in the sawdust. It could be that the production of various enzymes during the vegetative and reproductive phases, helped to solubilize the lignin and degrade the cellulose which were later absorbed by the mushroom mycelium for the production of fruit bodies (Belewu and Belewu, 2005). Mushroom sample grown on SBL substrate had an exceptionally high level ($p< 0.05$) of protein content of 3.98%wwb, confirming previous research reports (Belewu and Belewu, 2005; Khare et al., 2006) that oyster mushrooms grown on banana leave do contain high protein value. They attributed this to the addition of fungal proteins during solubilization and degradation of lignin. Belewu and Belewu (2005) further stated that the extra cellular enzymes secreted by the mushroom mycelium, contain amorphous homo and heteropolysaccharides which is often in association with mushroom protein. In support of this is the report of Ahmed et al. (2009) that nitrogen content in fruiting bodies was higher in mushroom grown on nitrogen rich substrates. The low protein level of mushroom harvested from CPO, SDO and SDW substrates could therefore, be as a result of the poor nitrogen level of the substrates. The fat content obtained in this research were generally low (0.15-1.83%), thus agreeing with the report of Tripathi (2005) and Chinda and Chinda (2007) that mushroom are low sources of dietary fat.

Effect of substrate type on micronutrients content of oyster mushroom

Substrate used in the cultivation of the mushroom

Table 4. Proximate composition of oyster mushroom cultivated on different substrates*.

Substrate	Parameter (%)				
	Moisture	Ash	Fiber	Protein	Fat
SDO	89.38 [a] ±2.8	1.27 [a] ±0.56	1.75 [e] ± 0.28	3.00 [c] ±0.16	0.26 [d] ±0.06
SDR	89.17 [b] ±1.7	0.25[d] ±0.04	2.28 [c] ±0.47	2.99 [c] ±0.15	0.15 [e] ±0.03
SDW	86.60 [b] ±0.8	1.27 ±0.50[a]	1.80 ±0.12[e]	2.11 ±0.11[e]	0.40 ±0.13[c]
SBL	72.23 [d] ±2.2	0.44 ±0.21[c]	3.18 ±0.05[a]	3.98 ±0.05[a]	1.83 ±0.02[a]
SDC	81.80 [c] ±0.5	0.73 ±0.13[b]	3.95 ±1.99[a]	3.15±0.034[b]	0.66±0.15[b]
CPO	87.30 [b] ±1.4	0.79 ± 0.12[b]	1.69 ± 0.19[e]	2.73 ± 0.19[e]	0.15 ± 0.02[e]
LSD	**1.17**	**0.11**	**0.15**	**0.10**	**0.10**

*Values with different superscript on the same columns are significantly different. Data are mean of three readings, and on wet weight basis. SDO = Sawdust only, SDR = Sawdust + Rice bran + $CaCO_3$, , SDW = Sawdust + Corn waste + $CaCO_3$, , SBL = Sawdust + Banana leaves, SDC = Sawdust + Cassava peel + $CaCO_3$, , CPO = Cassava peel only. Values are means of duplicates ± standard deviation.

Table 5. Micro nutrients content of oyster mushrooms grown on different substrates*.

Mineral (mg/100 g)	Substrates						LSD
	CPO	SBL	SDO	SDC	SDR	SDW	
Magnesium	2.32 [f] ±0.81	3.30 [c] ±0.82	3.45 [b] ±0.45	2.67 [e] ±0.42	3.13 [d] ±0.97	4.09 [a] ±0.87	**0.02**
Calcium	5.39 [c] ±0.81	6.64 [a] ±0.29	4.58 [e] ±1.62	4.79 [d] ±0.67	4.09 [f] ±0.18	6.54 [b] ±0.76	**0.04**
Potassium	0.66 [e] ±0.02	1.46 [b] ±0.26	1.41 [c] ±0.29	0.85 [d] ±0.13	1.57 [a] ±0.50	1.39 [c] ±0.51	**0.36**
Sodium	16.21[e] ±0.9	20.55 [b] ±2.8	20.48[c]±4.03	16.81 [d] ±1.12	21.23 [a] ±1.34	21.23 [a] ±1.78	**0.02**
Manganese	3.19 [f] ±1.23	4.51[b] ±0.95	4.39 [c] ±0.92	3.27 [e] ±0.21	4.13 [d] ±0.87	4.81 [a] ±0.11	**0.02**
Iron	1.93 [e] ±0.05	2.67 [b] ±0.06	1.96 [c] ±0.05	1.95 [d] ±0.12	1.87 [f] ±0.98	2.97 [a] ±0.13	**0.005**
Cupper	0.37 [f] ±0.01	0.52 [b]±0.19	0.47[c] ±0.01	0.39 [e] ±0.10	0.43 [d] ±0.01	0.53 [a] ±0.11	**0.007**
Zinc	0.75 [a] ±0.01	0.52 [f]±0.04	0.63 [c] ±0.04	0.65 [b] ±0.01	0.59 [d] ±0.09	0.53 [e] ±0.04	**0.003**
Vitamin B1	0.54[d]±0.01	0.54[d]±0.04	0.61[a]±0.08	0.56[c]±0.06	0.58[b]±0.05	0.58[b]±0.02	**0.02**
Vitamin C)	1.76[b]±0.05	3.14[a]±0.01	1.59[c]±0.01	1.74[b]±0.03	1.61[c]±0.04	3.12[a]±0.07	**0.49**

Values with different superscript on the same columns are significantly different. *Data are mean of three readings, and on wet weight basis. SDO = Sawdust only, SDR = Sawdust + Rice bran + $CaCO_3$, SDW = Sawdust + Corn waste + $CaCO_3$, SBL = Sawdust + Banana leaves, SDC = Sawdust + Cassava peel + $CaCO_3$,, CPO = Cassava peel only. Values are means of duplicates ± standard deviation.

significantly (p<.0.05) affected the mineral content of harvested mushroom (Table 5). For example the highest manganese (4.8 mg/100 g) and Iron (2.97 mg/100 g) contents occurred in oyster mushroom harvested from SDW substrate. Mushroom harvested from SDR had the lowest iron content of 1.9 mg/100 g while that harvested from CPO had the lowest manganese content of 3.19 mg/100 g. The variations observed in the mineral content of oyster mushroom in this study may be due to the difference in the biological and chemical composition of the substrate media (Abdurrahman et al., 2009; Ahmed et al., 2009). According to Oei (2003), Tripathi (2005), and Ahmed et al. (2009), mushrooms derive their food from the substrate on which they grow hence the observed variations in the mineral composition of the mushrooms grown on different substrates. However, the data from this research demonstrate that cultivated mushroom could be a good source of many dietary minerals.

The contents of vitamin B and C content of cultivated oyster mushroom, varied in the ranges of 0.54 to 0.61 mg/100g and 1.59 to 3.14 mg/100 g respectively. This result is in agreement with the findings of Chinda and Chinda (2007) and USDA Nutrient Data (2009). The highest (3.14 mg/100 g) vitamin C content was recorded on SBL substrate followed closely by and SDW substrates (3.12 mg/100 g) while the lowest (1.59 mg/100 g) was recorded on SDO substrate. The variations in the vitamin content of oyster mushroom are likely due to the variations in substrate composition (Vimla and Sundeh, 2005).

Conclusion

This study has demonstrated that some agricultural waste namely sawdust, cassava peel, banana leaves and rice bran can be used effectively for cultivation of oyster mushroom and that the nutritional value of the

domestically grown oyster mushroom were greatly affected by the substrate media. The implication of these findings is that substrates could be tailored to achieve desired mushroom yield and nutrient profile. Despite the differences in chemical composition of the mushrooms, the overall result indicated that fruit bodies of domestically cultivated mushroom had nutrient qualities similar to other exotic mushrooms. It is worthy of note also that the domestically cultivated mushrooms had higher protein content than some cereals and vegetables. This study has proven that commercial cultivation of mushrooms is feasible given the abundance of agricultural waste in Nigeria. Mushroom cultivation will create job opportunities in Nigeria and equally create avenue of utilizing agricultural waste materials. The government and other food supply stake-holders can redirect majority of our agricultural waste into mushroom growth substrate. This will not only provide an economical gain and protection to the environment but will also be a source of provide a nutritious food.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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