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Original Research Article

# Effects of *Khaya Ivorensis* and *Hevea Brasiliensis* Wood Sawdust on the Cultivation and Nutritional Composition of *Pleurotus Ostreatus* (*Jacaum ex. Fr. Kummar*)

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**Abstract**: This study was conducted to examine the effect of hardwood (Khaya ivorensis) and softwood (Hevea brasiliensis) on the proximate and nutritional composition of (Pleurotus ostreatus) (Jacaum ex. Fr. Kummar) with the aim of comparing its proximate and nutritional composition on the soft and hardwood substrates. Completely Randomized Design (CRD) was used by cultivating P.ostreatus on substrates prepared from Khaya ivorensis and Hevea brasiliensis sawdust, evaluating growth yield parameters, analyzing the harvested mushrooms for proximate and nutritional composition, and statistically comparing the results using ANOVA at 5% significance level. The proximate composition and mineral content of the harvested Pleurotus ostreatus were analyzed from the two substrates. The proximate composition result showed that softwood contains more crude fibre at 56.31%, while hardwood contain 43.29% of crude fibre. Furthermore, softwood contains 24.05% of carbohydrates and 19.82% of carbohydrates in hardwood. The table further shows that crude lipid is the least (11.74%) in softwood and 8.13% in hardwood. While ash content is high in hardwood, with 0.42 and 0.21 in softwood. There is no significant difference ($p>0.05$) between the percentage nutrient content in hardwood and softwood, however there is a statistical difference $p<0.05$) in the minerals constituent among the wood types, with the coefficient of determination at 0.823, meaning that 82.3 % of the data influenced this result. The result of the micro nutrients analysis indicates that hardwood mushrooms contain more minerals compared to softwood mushrooms; however, potassium and phosphorus are higher at 1740.8 and 520.37 mg/100g, respectively. The least mineral happens to be vitamin C for both softwood and hardwood mushrooms at 12.96 and 13.54 mg/100g, respectively. There is no significant difference ($p>0.05$) between the percentage of micro nutrients in hardwood and softwood, however there is a statistical

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difference $p<0.05$) in the minerals constituent among the wood types, with the coefficient of determination at 0.984, meaning that 0.961% of the data influenced this result.

**Keywords**: Mushroom Substrate, Proximate Composition, Nutritional Composition, Pleurotus Ostreatus

## INTRODUCTION

Oyster Mushroom (OM) is among over forty (40) species of mushrooms which belong to the genus *Pleurotus ostreatus*. Little interest has been shown in the cultivation of Oyster mushrooms in Nigeria until recently. Mushroom production and products is gaining more ground globally and in particular in Nigeria. For centuries, many traditional diets have been prepared in Nigerian communities, using different kinds of mushrooms, including Oyster Mushrooms of *Pleurotus ostreatus, Pleurotus eryngii* and *Pleurotus pulmonarius* strains, among others. Different types of mushrooms are found in the wild in the southern part of Nigeria, and the indigenous populations of the region are known to consume them. Mushrooms have been used as a food supplement for centuries, not only for their flavour, aroma and nutritive values but also for their medicinal properties (Patel *et al*, 2012; Deepalakshmi & Mirunalini, 2014). Mushrooms have been reported to have high culinary values due to their high-quality proteins, vitamins, fibres and many medicinal properties, and are therefore classified as nutraceuticals (Patel *et al,* 2012). So many factors affect the nutritional composition, growth and yields of mushrooms. They include different strains, methods of cultivation, stage of harvesting, composition of growth substrates and environmental factors such as temperature and relative humidity (Ogundele *et al.,* 2017). Cultivation of *Pleurotus ostreatus* helps in managing organic waste, whose disposal has become a problem in many cases. Its cultivation and consumption will help in providing solutions to the malnutrition problem in developing countries (Patience *et al.* 2018) and jobs for people. When the environmental conditions are good (temperature, relative humidity, luminosity), they produce lignocellulase enzymes (laccase and Manganese Peroxidase) which convert these lignocellulosic residues into food (Sánchez, 2010). It has been reported that the consumption of Oyster Mushrooms elicits immuno-modulatory and immuno-stimulatory effects in humans, and boosts immunity and improves its regulation, because of the presence of high concentrations of Fe, and enhances RBC count (Gbolagade *et al.,* 2016). Research has shown that the consumption of Oyster Mushrooms elicits anti-inflammatory actions, boosts and improves mood, and strengthens bones due to the presence of Vitamin D in significant concentration (Ogundele *et al.,* 2014). Oyster mushrooms have also been reported to possess anti-inflammatory effects within the body by down-regulating pro-inflammatory pathways involved in both chronic (autoimmune) and acute (allergies, tissue injury-type) hyperinflammatory conditions (Patel *et al.*,2012). Many research works have been conducted to determine the nutritional composition and the yield of *Pleurotus ostreatus* grown on various substrates such as awdust from *Daniella oliveri tree* (Ogundele *et al.,* 2014), straw and rice hull (Das and Mukherjee, 2017).

Sawdust from softwood has been reported to give the highest yield (Pathmashini *et al*., 2008). However, further work is required to determine the nutritional composition, yield and mineral content of *Pleurotus ostreatus* grown on other substrates such as sawdust from hardwood. Hence, the study is aimed at comparing the mineral composition and proximate composition of *Pleurotus ostreatus* grown on sawdust from both soft (*Havea brizilensis*) and hardwood (*Khaya ivorensis*).

## MATERIALS AND METHODS

### Study Area

This research was carried out at the Demonstration Farm (Mushroom unit), Faculty of Agriculture, University of Port Harcourt, Rivers State. The farm lies on a latitude of 4.5N and longitude 7 ° E on an elevation of 18 meter above seas level, a mean annual temperature of 27$^{o}$C and rainfall of 2000-2476mm (Naraian *et al*., 2010).

### Sources of Wood Substrates

The logs of various wood species were collected from Timber market at Rumuosi in Port Harcourt, Rivers State (Uniport) and was sawn into sawdust and weighed at a volume of 5kg.

| | **Hardwood species** | **Scientific name** |
|---|---|---|
| a. | Mahogany | *Khaya ivorensis* |
| | **Softwood species** | **Scientific name** |
| a. | Rubber tree | *Hevea brasiliensis* |

### Mycelia Preparation (Spawning)

The 200g of Irish potato was peeled, boiled in 500ml of water and 50g of agar powder and 50g of glucose D were added to the water and sterilized for 15 — 20 minutes in a conical flask. The medium (PDA) was poured into a well cleaned sterilized petrish discs. Upon cooling, a tissue of the mature *Pleurotus ostreatus* fruiting body was cut and inserted into petrish discs containing the Potato Dextrose Agar. After 3 to 4 days, the mycelia of *P. ostreatus* was seen growing from the cultured tissues in the petrish disc.

### Multiplication of *P. ostreatus* Mycelia

Guinea corn (sorghum) of 200g was measured and washed thoroughly to eradicate the non-viable grains that were floating on the water surface. The seeds were boiled for 1hr until they were soft, the grains were air-dried for 2 hours in order to reduce the water content of the grains which might affect the growth of the mycelia. After drying, the grains were poured into a transparent dried vita milk bottle covered with foil paper and sterilized for one hour and thirty minutes to get rid of all microbes that might hinder the growth of the mycelia (Muhammed *et al.,* 2007).

Upon cooling, a cleaned and sterilized surgical blade was used to cut parts of the mycelia and drop on 30cl bottles of the grains and spawn were reproduced or replicated.

**Preparation of Different Substrates**

The *Khaya ivorensis* sawdust (3kg) was mixed with 0.3kg of wheat bran, 0.06kg of calcium trioxo carbonde (CaCO3) and 1litre of water distilled in order to boost the growth of the mycelia. The lime (CaCO3) controlled the pH level of the substrates while water moistened the substrates to enhance mycelia growth. Blocks of each substrate sample (1kg) was made by putting the substrate in a long 25cm heat resistance thick polythene bag. The transparent bag identified the substrates that were contaminated and fully colonized. After bagging, sterilization by heating was carried out on the various substrates for 3-4 hours to eliminate germs and microbes which affected the growth of the mycelia (Muhammad *et al.,* 2007). The substrates were loaded in a sterilization drum using 50kg gas cylinder as a source of heat. The heated samples were allowed to cool and inoculated with *P. ostreatus* mycelia (Tesfaw *et al*., 2015).

**Substrates Innoculation Procedure**

The spawn of *P. ostreatus* produced and multiplied was inoculated into the sterilized substrate samples. 50g of the spawn was used to inoculate each 1kg block of the different substrates. The laboratory was cleaned and sterilized with 400ml of methylated spirit and 500ml diluted ethanol to fight bacterial contamination of the spawn and the substrates.

**Colonization of Substrates**

The different substrates inoculated were transferred and stacked on shelves in the incubation room where the growth of the *P. ostreatus* mycelia take place on the substrates (ie colonization of substrate). Full colonization of the different substrates take place within an interval of 3-4 weeks from the day of inoculation.

**Determination of Morphology Parameters**

**Cap diameter:** This was determined by placing a transparent ruler across the center of the pileus of each harvested mushroom fruit body and reading off the diameter

**Stripe length:** This was determined by placing a transparent ruler along the length of each fruit body stripe.

**Weight of fresh fruit bodies.** This was determined by weighing each fresh fruit body immediately after harvest using a portable digital balance.

**Determination of Total Phosphorus, Magnesium, Iron, Calcium, Sodium, Potassium**

The standard method for Total phosphorus TP determination in water and wastewater by the Water Environment Federation (Method 4500-P).

**Procedure**

70µL of the 250 g $L^{-1}$ $Na_2S_2O_8$ solution was added to a brown glass bottle containing 25 mL of the aqueous sample and mixed.

These bottles were placed in the water boiler at 95°C for 3h.

After digestion, 0.5 ml of the Ascorbic Acid AA and mixed reagent MR solutions were added sequentially to the cooled digested solution, and mixed thoroughly.

The characteristic blue colour hilly developed within 5 min at room temperature.

The absorbance of the formed PMB compound was measured at 700 nm with a spectrophotometer using a 5-cm cuvette.

The procedure for acidic and alkaline digestions are the same as that for the neutral condition except that the $Na_2S_2O_8$ solution was prepared with acid or base accordingly. Both acidic and alkaline digestion processes were strictly according to the standard oxidation methods (Hansen & Koroleff, 1999).

**Vitamin C Ascorbic Acid Procedure**

2 ml of the standard, sample and blank solution was taken in 25 ml volumetric flask. In each volumetric flask, 2ml sulphuric acid (10% v/v) and 5 ml ammonium molybdate (10% w/v) added and mixed well, then kept for 50 minute at room temperature. It was diluted 25 ml with distilled water and the absorbance was recorded at 450 nm against a blank

**Sample Preparation**

**Wet digestion method:** a total volume of 100mL of $H_2SO_4$, $HNO_3$ and HCIO in the ratio of 40%:40%:20% was mixed together

1) Weigh 1-3g of the sample into a conical flask.

2) Take 2m1 of the mixed acid to each of the sample in the conical flask.

3) Digest in a fume cupboard with hot plate until white fumes appear.

4) Cool and filter into a 100ml volumetric flask and make up to mark with distilled water.

**Operation or Procedure**

Because of differences among makes and models of AASs, it is not possible to formulate instructions applicable to every instrument. Follow the manufacturer's instructions, but in general proceed as follows:

1) Install a hollow cathode lamp for the desired metal.

2) Set the wavelength dial as specified by the analytical methodology.

3) Set slit width according to the manufacturer's suggested setting.

4) Turn on the instrument, apply the hollow cathode lamp current suggested by the manufacturer, and let the instrument warm up until energy sources stabilize, usually about 10 to 20 minutes.

5) Readjust current if necessary after warm-up. Adjust wavelength daily until optimum energy gain is obtained.

6) Align the lamp in accordance with the manufacturer's instructions.

7) Install a suitable burner head and adjust its position. A 10cm, single-slot burner head is recommended for air-acetylene flames.

8) Turn on the air, and adjust the flow rate according to the manufacturer's instructions to give maximum sensitivity for the metal being measured.

9) Turn on acetylene and adjust the flow rate to the value specified.

10) Ignite the flame and let it stabilize for a few minutes.

11) Aspirate blank and zero instrument.

12) Aspirate a standard solution and adjust the aspiration rate of the nebulizer to obtain maximum sensitivity.

13) Adjust the burner both vertically and horizontally to obtain maximum response.

14) Aspirate blank again and re-zero the instrument.

15) Aspirate a standard with a concentration near the middle of the linear range and record absorbance.

16) The instrument is now ready to operate.

17) When analysis is finished, extinguish flame by turning off acetylene first and then air.

**Experimental Design/Data Analysis**

The experimental design used is a Completely Randomized Design with two treatments replicated five times. The data collected was subjected to one-way ANOVA and DUNCAN Multiple Range Test (DMRT) to separate the means where significant differences exist. Inferential and descriptive statistics were used to analyse the data.

**RESULTS**

**Proximate composition of the soft and hardwood substrates used in the experiment.**

Figure 1 indicates that softwood contains more crude fibre at 56.31%, while hardwood contain 43.29% of crude fibre. Furthermore, softwood contains 24.05% of carbohydrates and 19.82% of carbohydrates in hardwood. The table further shows that crude lipid is the least (11.74%) in softwood and 8.13% in hardwood. While ash content is high in hardwood with 0.42 and 0.21 in softwood. There is no significant difference ($p>0.05$) between the percentage nutrients content in hardwood and softwood, however, there is a statistical difference $p<0.05$) in the minerals constituent among the wood type as shown in table 4.1 below with the coefficient of determination at 0.823 meaning that 82.3 % of the data influenced this result (Table 1).

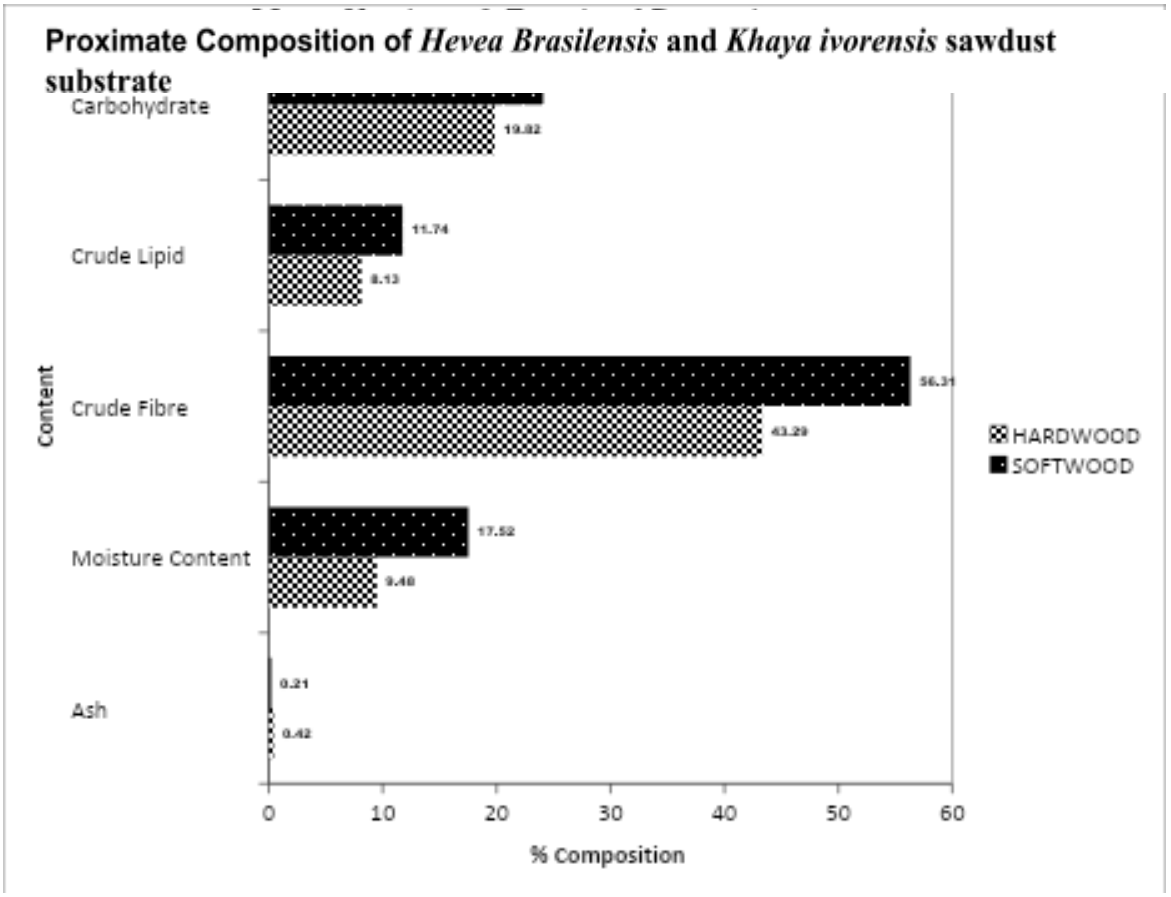


**Figure 1.** Proximate composition of the soft and hardwood substrates used in the experiment

**Table 1.** ANOVA result for wood type and micro nutrient constituents

| Source | Type III Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Wood Type | 55967.402 | 1 | 55967.402 | 1.305 | 0.297 |
| Mineral Constituents | 2844192.184 | 6 | 474032.031 | 11.057 | 0.005 |
| Error | 257226.054 | 6 | 42871.009 | | |
| Total | 3157385.640 | 13 | | | |

a. R Squared = 0.919 (Adjusted R Squared = 0.823)

**Nutritional Components of the Harvested *P. ostreatus* on the soft and hard wood substrates**

The result of the micro nutrients analysis as shown in figure 4.2 indicates that hard wood mushroom contain more minerals compare to softwood mushroom, however, potassium and phosphorous are higher at 1740.8 and 520.37 mg/100g respectively. The least mineral happens to be vitamin C for both soft wood and hard wood mushrooms at 12.96 and 13.54 mg/100g respectively There is no significant difference ($p>0.05$) between

the percentage micro nutrients in hard wood and soft wood, however there is a statistical difference p<0.05) in the minerals constituent among the wood type as shown in Table 4.3 below with the coefficient of determination at 0.984 meaning that 0.961% of the data influenced this result.

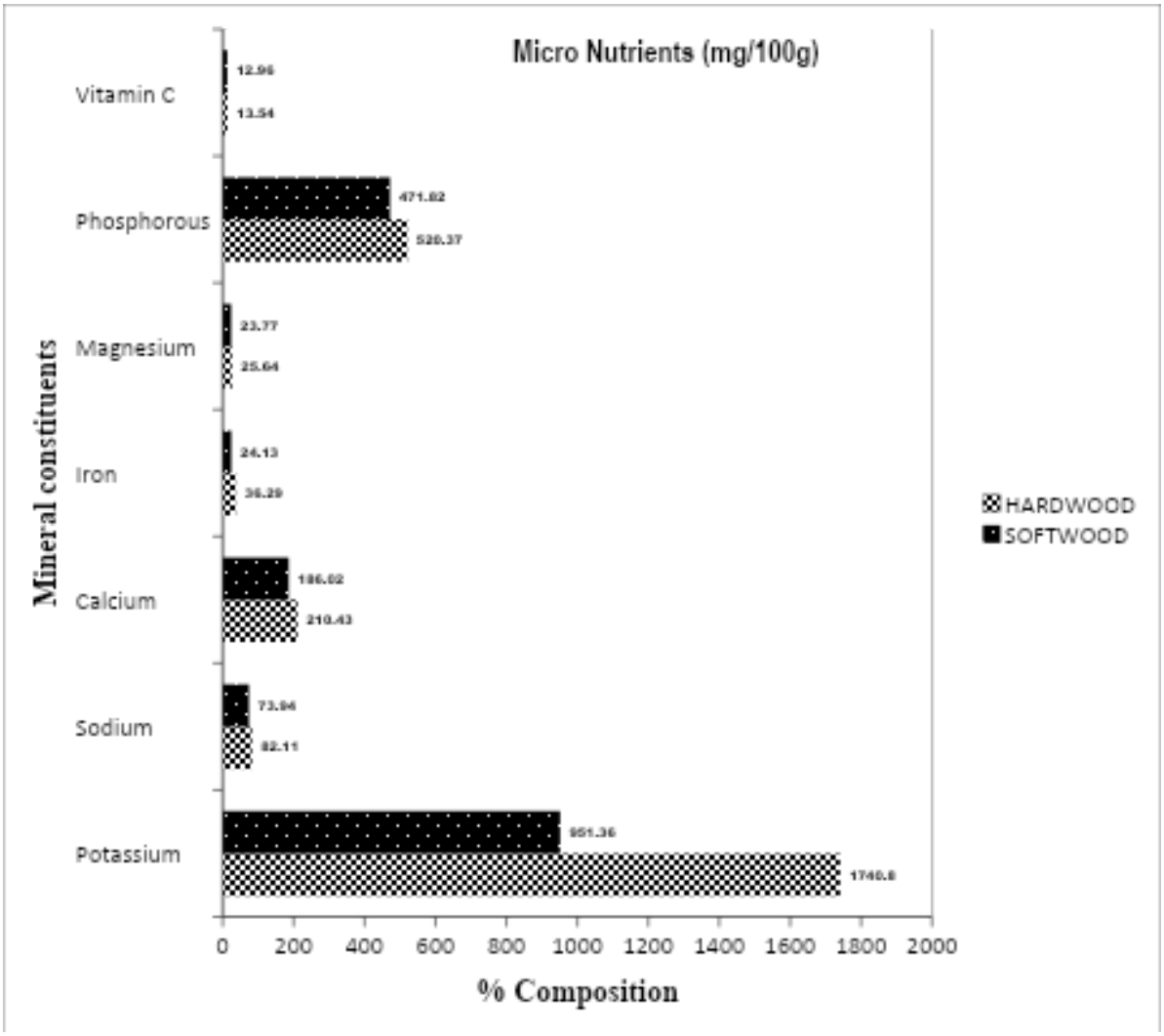


**Figure 2.** Nutritional Components of the Harvested *P. ostreatus* on the Soft and Hard Wood Substrates

**Table 2.** ANOVA Result for Macro Nutrients and Wood Type

| Source | Type III Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Macro Nutrients | 1672.801 | 2 | 836.400 | 60.569 | 0.016 |
| Wood type | 72.732 | 1 | 72.732 | 5.267 | 0.149 |

| | | | | | |
|---|---|---|---|---|---|
| Error | 27.618 | 2 | 13.809 | | |
| Total | 1773.151 | 5 | | | |
| a. R Squared = 0.984 (Adjusted R Squared = 0.961) | | | | | |

There is a statistical difference in the macro element content $p<0.05$, however, for wood type, there is no significant difference $p>0.05$.

## DISCUSSION

### Nutritional Composition of the *P. ostreatus* Harvested from Soft and Hard Wood Substrates

The mineral contents of *Pleurotus ostreatus* harvested from the two substrates used (Hardwood and softwood sawdust) are shown in Figure 1. Calcium, Sodium, Potassium, Phosphorus, and Magnesium were analyzed. In this study, Potassium have the highest percentage value (1740.8%) in hard wood substrate, and (951.36 %) in soft wood, followed by Phosphorus (520.37%) in hardwood substrate, and (471.82 %) in soft wood substrate, Calcium (210.43%) in hardwood substrate, and (186.02%) in softwood substrate, Sodium (82.11% and 73.94%) in hard and soft wood respectively, Iron (36.29% and 24.13%) in hard and soft wood respectively, and Vitamin C (13.54% and 12.96%) for mushroom harvested from hardwood and softwood substrates, this is almost similar to the findings of (Ogundele *et al*., 2017), who reported in his study that, Potassium have the highest value (22.81 mg/100 g and 21.90 mg/100 g), followed by Phosphorus (10.36 and 10.09 mg/100 g), Calcium (3.51 and 3.42 mg/100 g), and Sodium (3.51 and 3.00 mg/100 g) for mushroom harvested from hardwood and softwood substrates. The results of the nutritional composition of the harvested *P. ostreatus* on the soft and hard wood substrate indicate that mushrooms harvested from hard wood contains more minerals compare to mushrooms harvested from softwood substrates. Softwood contains more of the marco nutrients than the hardwood, except the ash content which is higher in hardwood with relative abundance of 0.42% compare to the soft wood with 0.21% composition. The result also indicates that the minerals composition of harvested mushrooms (micro-nutrients) were higher in hardwood compared to the mushrooms from softwood. This study shows that *Pleurotus ostreatus* growth performance were higher on softwood substrates (*Hevea brasiliensis*) than the hardwood *(Khaya ivorensis*), as it contains of the macro nutrients than the micro nutrients. It is therefore ideal to use more of the softwood substrates (*Hevea brasiliensis*) for the cultivation of *P. ostreatus* than the hardwood (*Khaya ivorensis*)

### Proximate Composition of the Soft and Hard Wood Sawdust

A true understanding and suitable management techniques of various trees and wood types (soft and hard wood) would definitely lead to high growth and productivity of mushrooms. Result of the study shows that *Hevea brasiliensis* sawdust substrate (softwood) contains more carbohydrate than Khaya ivorensis sawdust substrate (hardwood). Though, the ash content is higher in Khaya ivorensis sawdust substrates (hardwood) than the *Hevea brasiliensis* sawdust substrate (softwood) which is in line with Petterson (1984) ash

composition for US hardwood and softwood. The ash content is higher in hardwood than softwood (Petterson, 1984). The frequency of watering per week were higher in the *Khaya ivorensis* sawdust substrate than in *Hevea brasiliensis* sawdust substrate as the *Khaya ivorensis* absorb little moisture than *Hevea brasiliensis* (HB) which affect the morphology and physiological parameters of *P. ostreatus* (Chukunda *et al.,* 2017). The results of the nutritional components of the harvested *P. ostreatus* on the soft and hard wood substrates indicate that mushrooms harvested from hard wood contain more minerals compared to mushrooms harvested from softwood substrates. The relatively high percentage of calcium, vitamin C, phosphorus, magnesium, iron, sodium, and potassium in *Pleurotes ostreatus* harvested for *Khaya ivorensis* sawdust substrate and *Hevea brasiliensis* sawdust substrate samples proved that *P. ostreatus* is nutritious and good for human consumption. This is in agreement with the report obtained by Marlow (2001). On the contrary, the high moisture content of the fresh mushroom obtained cannot be kept for a longer time. This may be due to high water activity, which favoured microbial growth (Aletor, 1995).

## CONCLUSION

The result shows that *Hevea brasiliensis* substrates contain more carbohydrates with a relative abundance of 24.05%, while *Khaya ivorensis* contain 19.82%. Softwood contains more of the macro nutrients than the hardwood, except the ash content, which is higher in hardwood with a relative abundance of 0.42% compare to the softwood with 0.21% composition.

The result also indicates that the mineral composition of harvested mushrooms (micro-nutrients) was higher in hardwood compared to the mushrooms from softwood. This study shows that *Pleurotus ostreatus* growth performance was higher on softwood substrates (*Hevea brasiliensis*) than on hardwood *(Khaya ivorensis*), as it contains more macro nutrients than micro nutrients.

It is therefore ideal to use more of the softwood substrates (*Hevea brasiliensis*) for the cultivation of *P. ostreatus* than the hardwood (*Khaya ivorensis*).

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