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# Bio-safety Quality and Nutritional Status of *Pleurotus ostreatus* Cultivated on Sawdust of Two Selected Tropical Trees

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## ABSTRACT

*Pleurotus ostreatus* is an edible mushroom rated as the second most important in production in the world after *Agaricus bisporus*. The local availability of substrates made it an economically viable preposition in the tropics. Sawdust of two selected tropical trees: Hog plum (*Spondias mombin*, L; Family: Anacardiaceae) and African nut (*Pycnanthus angolensis*, Welw, Warb; Family: Myristicaceae, were used as the sole substrates for the cultivation of the mushroom. The mineral composition and proximate value of the harvested fruiting bodies were determined, also the toxicological effects on experimental animals (albino rats) was studied. Results obtained showed that the mushroom compared favorably with other food items in terms of its protein quality, carbohydrate and calorific values as it ranges from 7.54±0.14-11.74±0.29%, 43.65±0.32-50.67±0.41% and 273.40±0.16-307.54±0.13 kcal $g^{-1}$, respectively. The toxicological analysis through histopathological assay also revealed that there was no observable damage on the major organs of the experimental animals fed with mushroom composed diet (sample meal) and soybean composed diet (positive control). However, the organs of the experimental animals fed with protein free diet (negative control) were observed to have necrotic lesion and multifocal lymphocytic aggregates on the liver and kidney. Also, a disruption of the villi tips of the intestine was equally observed.

**Key words:** *Pleurotus ostreatus*, sawdust, *Spondias mombin*, *Pycnanthus angolensis*, nutrient composition, toxicological

## INTRODUCTION

Mushrooms are cultured worldwide for their taste, nutritional attributes and potential application in industries (Mata *et al*., 2005). Edible mushrooms are nutritionally endowed fungi mostly Basidiomycetes which grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Stamets, 2000; Lindequist *et al*., 2005; Iwalokun *et al*., 2007). Edibility of mushroom may be defined by criteria that include absence of poisonous effects on humans and desirable taste and aroma. *Pleurotus* species are a class of edible mushroom that has the capacity to convert nutritionally valueless substances into high protein food and are reputed to have a high saprophytic ability and to grow on a variety of cellulosic wastes (Yildiz *et al*., 2002).

*Pleurotus* species as one of the valuable and edible species of mushroom occupies the third place in the worldwide production of edible mushrooms, after *Agaricus bisporus* and *Lentinula edodes* (Chang, 1999). In recent years, it is reported to be the second most important mushrooms in production in the world of which 25% of the total world production of cultivated mushrooms are *Pleurotus*. *Pleurotus* are prospective source of valuable food protein with the ability to effectively bio-convert various lignocellulosic materials to protein (Wang *et al*., 2002). They are often called boneless vegetarian meat that contains 20-30% protein (dry weight) which is higher than those of vegetables and fruits and is of superior quality, they are of more valuable source of protein than beef or fish (Yildiz *et al.*, 2002).

The low availability of lignocellulosic materials from the family of Graminae for the cultivation of mushrooms in recent years has led to the search for an alternative source of raw materials as substrates for the cultivation of *Pleurotus* species. Attention has therefore been turned to the use of sawdust of tropical trees from our saw-mills which of course have been yielding tremendous results. In particular is *Spondias mombin*, a tropical tree which naturally supports the growth of the mushroom (Fakoya and Akinyele, 2008). The objective of this study was to determine the nutritional qualities and also assess the toxicological effects (if any) of consuming *Pleurotus ostreatus* cultivated on the sawdust of two selected tropical trees.

## MATERIALS AND METHODS

**Source of materials:** Pure spawn was obtained from the Research Laboratory of the Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, Nigeria.

**Preparation of substrates:** Sawdust of two tropical trees: Hog plum (*Spondias mombin*, L; Family: Anacardiaceae) and African nut (*Pycnanthus angolensis*, Welw, Warb; Family: Myristicaceae) were screened for any form of contaminants like stone, nails, pecks of wood and stick. The sawdust were packed into transparent plastic containers using the method of Fakoya and Akinyele (2008), the moisture content was maintained at 85% relative humidity while holes of 15 mm diameter were made at the centre and then sterilized by autoclaving at 121°C for 15 min.

**Cultivation of *Pleurotus ostreatus*:** Sterilized substrates were allowed to cool in an inoculating chamber where inoculation of the substrates with spawn was carried out. This was followed by incubation at 25±3°C in a box (1.0×0.8×0.5 m) made up of wood with an open and close roof system. The relative humidity, temperature and light intensity were monitored for the period of cultivation. The biomass of the fruiting bodies was monitored and recorded accordingly.

**Proximate and mineral compositions:** The proximate analysis was carried out according to the method described in AOAC (2005). Kjeldah's method was used to determine the total nitrogen while a factor of 6.25 was used to calculate the crude protein content (Wang *et al.*, 2002). The mineral contents was determined using Atomic Absorption Spectrophotometer after mineralization while the phosphorus content was determined using colorimetric methods.

**Bioassay:** Wistar strain albino rats weighing 25-8 g were purchased from the Department of Biochemistry, University of Ilorin, Nigeria and acclimatized for 2 weeks, during which period they were maintained *ad libitum* on commercial diet. The rats were subsequently divided into three treatment groups. Animals in group A were fed with protein free diet, while animals in group B were fed with the basal diet supplemented with soyabean meal and animals in groups C were fed with the basal diet containing mushroom as shown in Table 1. The feed and water were given *ad libitum* throughout the duration of feeding trial experiment; daily feed intake and weekly change in body weight were monitored throughout the experiment which lasted for 30 days. The albino rats were anaesthetized with chloroform and dissected while some major organs like the liver, kidney and small intestine were removed into 10% formalin for histopathological analysis using the method of Silva *et al.* (1999).

Table 1: Feed formulation for the evaluation of protein quality (g/1000 g)

| | Diets | | |
|---|---|---|---|
| Components | Protein free diet (g) | Soya bean composed diet (g) | Mushroom composed diet (g) |
| Cellulose | 40.0 | 40.0 | 40.0 |
| Sucrose | 100.0 | 100.0 | 100.0 |
| Corn oil | 40.0 | 40.0 | 40.0 |
| Min.mix/Vit mix | 50.0 | 50.0 | 50.0 |
| Methionine | 4.0 | 4.0 | 4.0 |
| Soya bean meal | - | 250.0 | - |
| Mushroom | - | - | 125.0 |
| Corn starch | 766.0 | 516.0 | 641.0 |
| Total | 1000.0 | 1000.0 | 1000.0 |

**Statistical analysis:** Quantitative data were expressed as mean ± standard deviation. Statistical evaluation of the data was performed using one-way analysis of variance followed by Duncan's multiple range test at 5% level of significance i.e., $p \leq 0.05$ (Zar, 1984).

## RESULTS

**Nutritional composition of *P. ostreatus* cultivated on the sawdust of *S. mombin* and *P. angolensis*:** Table 2 shows the nutritional composition of the cultivated *P. ostreatus* on the two selected tropical trees sawdust. The *P. ostreatus* obtained from *S. mombin* had higher calcium content of 11.40 mg/100 g while *P. ostreatus* obtained from *P. angolensis* had 5.80 mg/100 g. The iron contents were 0.14 and 0.05 mg/100 g for *S. mombin* and *P. angolensis*, respectively. For Sodium, Potassium and Phosphorus, it was 40.60, 28.60, 3086.50, 28.90, 25.40 and 2996.20 mg/100 g for *S. mombin* and *P. angolensis*, respectively. Also, *P. ostreatus* from *P. angolensis* had 11.74% protein content while *S. mombin* had 8.88%. The carbohydrate and calorific values were higher in *P. ostreatus* from *S. mombin* with the values as 50.67% and 300.11 kcal $g^{-1}$ while that of *P. angolensis* was 45.68% and 299.54 kcal $g^{-1}$, respectively. Table 3 shows the proximate composition of *P. ostreatus* when the duo was used at varied ratio composition, there were significant differences in the values of fat, carbohydrate and calorific values for the *P. ostreatus* when the composition of the duo sawdust were varied in ratio as the one with ratio 5a:5b gave the highest values as 8.39, 50.24% and 307.54 kcal $g^{-1}$, respectively.

**Nutrient utilization and growth performance of rats fed with different composed diets:** Table 4 showed the growth performance of rats fed with different composed diets. The protein free diet (negative control) had an average daily feed intake of 5.8 g/rat/day, daily weight gain of 0.95 g/rat/day) and feed gain ratio of 6.11 while soybean composed diet (positive control) and Mushroom composed diet (sample meal) gave an average daily feed intake of 5.8 and 6.0 g/rat/day, daily weight gain of 1.68 and 1.42 g/rat/day, feed gain ratio of 3.45 and 4.22, respectively.

**Histopathological analysis of rats fed with composed diets:** The histopathological observations of some organs namely: the liver, kidney and small intestine of albino rats fed with different composed diets are presented in Fig. 1-3 which showed that there was an observable pathological changes in the liver, kidney and the small intestine of the rats fed with the protein free diets when compared with the soybean composed diet (positive control) and the mushroom composed

Table 2: Nutritional composition of *P. ostreatus* cultivated on two different substrates

| Proximate composition | Substrates: *S. mombin* | *P. angolensis* |
|---|---|---|
| Moisture content (%) | 8.53±0.21[a] | 8.68±0.54[a] |
| Protein (%) | 8.88±0.18[a,b] | 11.74±0.29[b] |
| Fat (%) | 7.65±0.27[a] | 9.19±0.18[b] |
| Carbohydrate (%) | 50.67±0.41[b] | 45.68±0.11[a,b] |
| Crude fibre (%) | 18.34±0.25[a] | 21.26±0.26[a,b] |
| Ash content (%) | 5.93±0.09[a,b] | 3.45±0.34[a] |
| Calorific value (kcal $g^{-1}$) | 300.11±0.25[c] | 299.54±0.56[c] |
| Calcium | 11.40±1.34[f] | 5.80±1.88[e] |
| Iron | 0.14±0.04[e] | 0.05±0.01[d] |
| Sodium | 40.60±1.97[e] | 28.90±1.64[d] |
| Potassium | 28.60±0.48[d] | 25.40±0.13[d] |
| Phosphorus | 3086.50±1.08[e] | 2996.20±1.06[d] |

Values are means of triplicates±SD, Samples carrying the same superscripts in the same row are not significantly different at (p>0.05)

Table 3: Proximate composition of harvested *P. ostreatus* fruiting bodies cultivated on different substrate ratio (composition)

| Substrate's ratio | Moisture content (%) | Protein (%) | Ash (%) | Crude fibre (%) | Fat content (%) | Carbohydrate (%) | Calorific values (%) (kcal $g^{-1}$) |
|---|---|---|---|---|---|---|---|
| 1a: 9b | 12.01±0.21[b,c] | 7.54±0.14[a] | 8.11±0.19[b,c] | 18.55±0.22[a,b] | 7.68±0.19[a,b] | 46.11±0.13[a,b] | 277.70±0.16[a] |
| 2a: 8b | 11.18±0.14[b,c] | 8.86±0.26[a,b] | 9.83±0.20[c] | 17.14±0.18[a] | 8.02±0.21[b] | 44.97±0.11a,b | 279.21±0.22[a] |
| 3a: 7b | 10.88±0.26[b] | 9.49±0.36[b] | 8.87±0.21[b,c] | 19.32±0.16[b] | 7.79±0.20[a,b] | 43.65±0.32[a] | 273.40±0.16[a] |
| 4a: 6b | 13.31±0.11[c] | 10.28±0.13[c] | 7.11±0.09[b] | 18.23±0.19[a,b] | 6.91±0.12[a] | 44.16±0.18[a,b] | 270.24±0.15[a] |
| 5a: 5b | 8.08±0.18[a] | 10.04±0.19[c] | 5.02±0.11[a] | 18.26±0.21[a,b] | 8.39±0.22[b] | 50.24±0.11[c] | 307.54±0.13[c] |
| 6a: 4b | 8.79±0.20[a] | 9.68±0.23[b] | 6.93±0.15[a,b] | 18.89±0.28[a,b] | 6.99±0.18[a] | 48.72±0.18[b,c] | 288.49±0.21[b,c] |
| 7a: 3b | 9.49±0.18[a,b] | 9.83±0.14b | 5.83±0.23[a] | 19.39±0.12[b] | 7.41±0.26[a,b] | 48.05±0.21[b,c] | 289.58±0.16[b,c] |
| 8a: 2b | 10.64±0.42[b] | 8.47±0.35[a,b] | 6.78±0.30[a,b] | 18.59±0.23[a,b] | 8.41±0.24[b] | 47.11±0.22[b] | 290.45±0.33[b,c] |
| 9a: 1b | 9.44±0.32[a,b] | 10.18±0.23[c] | 7.41±0.17[b] | 18.11±0.21[a,b] | 6.14±0.24[a] | 48.72±0.19[b,c] | 282.69±0.25[b] |

a: *S. mombin*, b: *P. angolensis* Values are means of triplicates±SD, Samples carrying the same superscripts in the same column are not significantly different at (p>0.05)

Table 4: Nutrient utilization and growth performance of rats fed with different composed diets

| Sample | Average daily feed intake (g/rat/day) | Daily weight gain (g/rat/day) | Feed gain ratio |
|---|---|---|---|
| PFD | 5.8±0.35[a] | 0.95±0.01[c] | 6.11[b] |
| SCD | 5.8±0.15[a] | 1.68±0.01[b] | 3.45[a] |
| MCD | 6.0±0.41[a] | 1.42±0.01[a] | 4.22[a] |

Values are means of triplicates±SD, Samples carrying the same superscripts in the same column are not significantly different at (p>0.05). PFD: Protein free diet, SCD: Soyabean composed diet, MCD: Mushroom composed diet

diet (sample meal). The group of rats fed with protein free diets showed a visible necrotic lesion and multifocal lymphocytic aggregates in their livers and kidneys while villi tips disruption was observed in their small intestines.

## DISCUSSION

The proximate composition of harvested *P. ostreatus* fruit bodies on substrates at ratio (1: 1) of *S. mombin* and *P. angolensis* showed the highest carbohydrate content and calorific value of 50.21±0.11% and 307.54±0.13 kcal, respectively. It was therefore observed that the substrates were


Fig. 1(a-c): (a) Photomicrograph of the liver of experimental rats fed with protein free diet showing necrotic lesion (b) Photomicrograph of the liver of experimental rats fed with soybean composed diet (c) Photomicrograph of the liver of experimental animal fed with mushroom composed diet


Fig. 2(a-c): (a) Photomicrograph of the kidney of experimental animal fed with protein free diet showing necrotic lesion (b) Photomicrograph of the kidney of experimental animal fed with soybean composed diet (c) Photomicrograph of the kidney of experimental animal fed with mushroom composed diet


Fig. 3(a-c): (a) Photomicrograph of the Intestine of experimental animal fed with protein free diet showing disruption of villi tip (b) Photomicrograph of the Intestine of experimental animal fed with soybean composed diet (c) Photomicrograph of the Intestine of experimental animal fed with mushroom composed diet able to influence the nutritive and calorific values of the mushroom which increased considerably compared to substrate when used individually. Therefore, mushrooms should be considered as a weight-management food, especially as substitute for higher calorie and fat ingredients in recipes, or protein staples like meat. The mineral composition in terms of Phosphorus was 3086.50±1.08 and 2996.20±1.06 mg/100 g on *S. mombin* and *P. angolensis* respectively. It was however the most abundant mineral element in the harvested mushroom and the relatively high phosphorus content was quite similar to that given by the Standard Tables of Food Composition in Japan (STFC, 1982) and Yildiz *et al.* (1998) as 1061 and 1647.6 mg/100 g dry matter respectively. The value of mineral content in *P. ostreatus* therefore is a reflection of the mineral composition of the substrate used. Also, a variation in the proximate composition of the fruiting bodies harvested from various substrates used for the study was observed as the sawdust from *P. angolensis* produced mushroom with 11.74±0.29% crude protein content while *S. mombin* was 8.88±0.18%, Also the carbohydrate contents of harvested *Pleurotus ostreatus* on *S. mombin and P. angolensis* was 50.67±0.41 and 45.68±0.11, respectively and this results agrees with the findings of Ragunathan and Swaminathan (2002) that carbohydrate content of *Pleurotus ostreatus* ranges between 40.60-53.30%.

The effects of feeding growing rats with different based diet showed average daily weight gain of 0.95±0.01, 1.68±0.01 and 1.42±0.01 g for protein free diet, soybean composed diet and mushroom composed diet respectively. Since growth is indicated by weight gain and increase in size, weight lose or reduction in size (as obtained in the protein free diet) shows that the taste diet is either not supporting growth or that it has some interferences (Edet *et al.*, 2010).

The histopathological results show that there was a clinical signs of toxicity such as a necrotic lesion and multifocal lymphocytic aggregates which were observed in the tissues of the liver and kidney of rats fed with protein free diet and this was attributable to the damage done to the tissues of the liver and kidney. Also there was a disruption of the villi tips in the intestine of rats fed with protein free diet thereby slowing down the rate of food absorption and also causing haemorrhagic infections. However, the organs of rats fed with mushroom composed diet were all normal without any observable damage. In conclusion, *Pleurotus ostreatus* cultivated on *S. mombin* and *P. angolensis* is of high nutritive and calorific values. It also has no observable clinical sign of toxicity when fed to experimental rats which shows that it can be used to augment for the source of energy value in man.

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