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Evaluation of the Mycochemical and Proximate Composition of Oyster Mushrooms (*Pleurotus ostreatus* VAR *Florida,* (MONT) Singer. *and P. pulmonarius* (FR) QUEL*)* Grown on Bark of *Gmelina arborea* Roxb., Straw of *Saccharum officinarum* L. and Pods of *Delonix regia* (BOJ. Ex Hook.) RAF

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Abstract

Mushrooms are fungal fruit-bodies which have over successive years served as suitable source of protein, carbohydrates, minerals and vitamins. The fruit-bodies derive their nutrients from varieties of substrates including agro-wastes. This study was conducted to evaluate the potentials of using the pods of *Delonix regia*, straws of *Saccharum officinarum* and bark of *Gmelina arborea* in the production of nutritionally-rich edible oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus pulmonarius*. The experiment was carried out in three replicates in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The results of the investigations were analysed using Analysis of variance (ANOVA), and the means were separated using Least Significant Different (LSD) tool. The result of the myco-chemical analysis shows that fruit-bodies of *P. ostreatus var florida*, produced in *Saccharum officinale* straw, yielded the higher alkaloids (3.93±0.010g/100g) than those produced in *Gmelina+Delonix* (3.84±0.025g/100g) and *S. officinale+D. regia ,* (3.77±0.03g/100g). The trend was the same with Flavonoids, tannins, saponins and phenols from *S. officinale, G. aborea + D. regia and S. officinale + D. regia,* and *D. regia. Pleurotus pulmonarius* from the substrates contained low concentration of alkaloids but higher concentration of flavonoids, tannins, saponins and phenols. Generally the substrates yielded fruit-bodies that are rich in protein. However those from *S. officinale + D. regia* appeared richer in protein (28.44±0.03g/100g). The substrates showed encouraging potentials for use in the production of nutritionally-rich edible *P. ostreatus* and *P. pulmonarius,* with *S. officinale + D. regia* standing out as a more likely preferred substrates. There appears to be a synergism between the substrates of *S. officinale* and *D. regia*, since the mean of their individual performance is less than their performance when combined.

1 Introduction

Mushroom generated a lot of interest presently[1]. This is because many people now eat mushroom.Mushrooms are involved in medicine to cure diseases[2]. They are used for bioremediation purposes and for revenue generation. The therapeutic and nutritional values of mushrooms also account for the increased interest. Many mushrooms are edible, while some are poisonous.

Edible mushrooms lack poison, they have desirable taste and aroma. It may be difficult to identify edible mushrooms visually. This is because some edible mushrooms have poisonous look-alke counterparts. The poisonous mushrooms that look like

some edible ones can be found in some species of *Amanita* genus in particular, *Amanita phalloides*[3].

The use of mushroom as food in Nigeria is probably as old as civilization. The mushrooms here are usually gathered from the wild[4]. Mushrooms have been providing the natural way to food health since ages; they are considered as a delicacy and occupy a place between meat and vegetables from nutrition point of view[5].

Mushrooms are low in utilizable carbohydrate, calories, and sodium. The carbohydrate content of mushrooms is low and occurs in the mushroom in form of xylose, ribose, mannose, glucose, and mannitol[6]. Similarly, found out that some mushroom samples from Taiwan contained the following substances, glucose, myo-inositol, arabitol, tetrahaloses and manitol[7].

They are low in cholesterol and fat but high in fiber and protein. They are also rich in B vitamins to help maintain a healthy metabolism. Mushrooms are excellent source of potassium, a mineral that helps lower elevated blood pressure and reduces the risk of stroke.

The protein content of mushrooms, however, varied in different species[8,9]. in their work, involving *Agaricus bisporus*, white button mushroom, *Lentinual edodes,* and *Pleurotus ostreatus* reported varying protein (based on amino acid) contents. Similarly, Stamets (2003) reported that protein content of mushroom has been averaged to 20% of the dry mass, with crude protein being a maximum in *P. ostreatus* (27.23%) and Minimum *P. djamo* (24.83%) and *P. sajor-caju* (25%)[10].

Similarly, gave the protein content of four Nigerian mushrooms, *Auriculoria auricular, Pleurotus squarrosulus, P.tuber-regium* and *Russula spp* as ranging from 15.0 – 24.96 g/100g on the dry matter basis. Reported substantial quantities of myco-chemicals and minerals in some tropical edible mushrooms[11]. Mushroom contains appreciable quantities of mineral elements. Mushrooms contained minerals that are higher than the mineral contents of fish vegetables and meat[12].

2 Materials And Methods

2.1 Source of spawn

Spawn of *Pleurotus ostreatus* and *P. pulmonarius* used for this work were obtained from the mycology laboratory of Michael Okpara University of Agriculture Umudike (Fig 1a and 1b).

2.2 Substrates

The substrates (*Delonix regia dry pods, Gmelina arborea bark* and the sugarcane straw) used for this study were coliected from the surroundings of National Crop Research Institute (NACRI), Umudike, Aquada Cooperation, old Umuahia, Umuahia South, L.G.A of Abia State and Uzuakoli market, in Bende LGA of Abia State all in Nigeria, respectively.

Fig 1a: Spawn of *P. pulmonarious*

Fig 1b: Spawn of *P. ostreatus*

2.3 Substrates preparation

The above named substrates were chopped to about 2cm long[13]. Sugar cane straw and bark of *Gmelina* were separately soaked overnight in clean tap water according to the method of Sharma and Satish, 2014, while *Delonix* pods were soaked in clean tap water for 3 (three) days[14]. The substrates were dispensed separately in three replicates into uniformly perforated transparent 5-litres plastic buckets, and were pasteurized for 2hours at 80^0C in a gas-heated drum. The substrates were labeled as S (Sugar cane straw), D (*Delonix regia* pods), G (*Gmelina arborea* bark) with the substrate combinations as (S + D, G + D and G + S) having percentage mixture at 70% x 30% and 60% x 40%.

2.4 Inoculation of substrates

After cooling, the substrates were inoculated with grain spawn by placing equal quantity of the spawn in 3 to 4 layers of the substrate. The buckets were covered and kept in the wooden rack in the culture room.

2.5 Preparation of the cropping room

Before the incubation of the substrates the cropping or culture room was scrubbed with water and detergent. Cobwebs were

removed from walls and the room was disinfected with Dettol. It was also ventilated by opening the windows this will hopefully maintain the day time temperature at 18 to 30^{0}C and reduce carbon dioxide concentration of the rooms. To maintain high humidity which is required for good vegetative growth of the mushroom the room will be constantly flooded with clean tap water.

2.6 Growth characteristics and proximate composition of fruit-bodies grown on different substrates

Fruit-bodies were harvested from the various substrates. They were counted, and measurement of the fresh weight, pileus and stripe dimensions, were taken before they were stored in perforated paper bags and dried according[15] (Fig. 2a & 2b).

Fig 2a: Fruit-bodies of *Pleurotus pulmonarious*

Fig 2b: Fruit-bodies of *P. ostreatus*

2.7 Preparation of samples for analysis

For the determination of the proximate composition of the fruit-bodies of the mushroom harvested from different substrates, the mushrooms were oven dried at 104^{0}C for four hours following the method of[15]. About 500 g each of the dry samples were broken into sizable pieces (about 2 cm) and then finely ground into powder of particle size of less than 0.5 mm using a Thomas milling machine[10]. The resulting powder was stored in dry air-tight bottles until needed for analysis.

2.8 Moisture content determination

To obtain the moisture content value of the mushroom fruit-bodies, 5g of the powdered dry samples was placed on clean dry petri-dishes of known weight. This was placed in an electric oven at 75^{0}C and allowed to dry for 6-8hours[16].The oven-dried samples were then weighed to constant weight.

The percentage moisture of dry sample was consulate as follows:

$$\frac{\text{Weight of dry sample} - \text{fresh weight of sample}}{\text{Fresh weight of sample}} \times 100$$

2.9 Ash content of fruit bodies

To obtain the ash content of the fruit bodies, 5 g of the ground dry sample was used. This will be subjected to weighing before and after burning it at 500 ^{0}C in a furnace. The furnace was allowed to cool.

The crucible was transferred into desiccators and allowed to cool further, before it is weighed again[2].

The percentage (%) ash content is expressed as:

$$\frac{\text{Weight of crucible + lid + ash} - \text{weight of crucible lid}}{\text{Weight of sample}} \times 100$$

2.10 Dietary fiber

The total dietary fibre of the fruit-bodies was determined according to the method of Weende. Two grams (2 g) of the samples were put into a 250ml beaker and hydrolyzed by adding 20 ml of dilute sulphuric acid and boiling for about 30minutes on hot plates.

The mixture was filtered through a piece of white nylon cloth; then rinsed with hot distilled water. The residue was again boiled with 50ml of 2.5% sodium hydroxide (NaOH) for 30mins, filtered and rinsed with distilled water; the residue was finally collected and transferred into a crucible, dried in the oven to a constant weight. The sample was burned in a muffle furnace.

The weight of the fibre was calculated and expressed as percentage fibre as follows:

Crude fibre = Weight of dry sample + crucible – weight of crucible + ash

$$\%\text{Crude fibre} = \frac{\text{Weight of fibre}}{\text{Weight of sample}} \times \frac{100}{1}$$

2.11 Crude protein

Crude protein content of the sample was determined by the use of the Micro-kjeldahl method. By this method, total nitrogen

content was determined first and the value was multiplied by 6.25 coefficients[16].

The crude protein therefore = the total Nitrogen multiplied by 6.25.

2.12 Determination of fats and oils

Fat content was determined by the continuous solvent extraction gravimetric method using soxhlet apparatus. The method was described by many including. A measured weight of the sample 5 g was wrapped in a previously weighed porous paper (Whatman paper) and placed inside a soxhlet reflux flask, containing 300 ml of solvent (petroleum ether), whose upper end was connected to a condenser. The solvent boiled, vaporized and condensed into the reflux flask thereby covering the wrapped samples, on heating the solvent in the flask through an electric heating mantle. The samples remained with the solvent until the flask filled up and siphoned over, thus carrying its extracted oil to the boiling solvent. The cycle of boiling and vaporizing took about five hours. The solvent was recovered and defatted wrapped samples carefully retrieved using a pair of forceps. The defatted samples were dried in the oven at 80^0C for 30 mins, cooled in a desiccator and reweighed.

The weight of oil extracted (fat) was obtained and expressed as a percentage of the sample using the formular below.

$$\%fat = \frac{100}{1} \times \frac{W_2 - W_3}{W_2 - W_1}$$

Where: W_1 = weight of empty Whitman paper

W_2 = weight of paper and sample (wrapped) before defatting

W_3 = weight of paper and defatted sample after drying

2.15 Calculation of carbohydrate content of the sample

The carbohydrate contents of dry samples of *P. ostreatus* and *P. pulmonarium* was calculated as follows:

CHO (%) dry weight = 100-cp+ fat + ash + TMC + DF.C in g/100g DW[15]

where

CHO = Carbohydrate; CP = Crude protein; FO = Fats and oil; MC = Moisture content and DF =Dietary fibre.

2.16 Determination of the Myco-chemcial composition of the fruit bodies of P. Ostratus var florida and P. Pulmonarius produced from different substrate

2.16.1 Determination of alkaloids

Five grams of the dry powdered sample were weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for four hours. This was filtered and the extract was concentrated by evaporating in boiling water to one quarter of the original volume. Concentrated Ammonium hydroxide was added drop-wise to the extract until the precipitation was complete.

The suspension was allowed to settle and the precipitate was collected by filtration and weighed[17]. The alkaloids are expressed as percentage thus:

$$\% \text{ alkaloids} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times \frac{100}{1}$$

2.16.2 Determination of flavonoids

Five grams of the sample was extracted repeatedly with 10ml of 80% aqueous methanol at room temperature. The solution was filtered through Whitman filter paper No.42 (125 mm). The filtrate was transferred into a crucible of known weight, evaporated to dryness over a water bath, and dried in an electric oven to a constant weight[18].

The flavonoids was expressed as a percentage

Thus:

$$\% \text{ flavonoids} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times \frac{100}{1}$$

2.16.3 Determination of phenol content

To determine the phenol content of the powdered sample of the mushroom, a fat-free sample was used. About 2 g of the sample was defatted with 100ml of diethyl either for 15 minutes. Five milliliter of the extract was pipetted into a 50 ml flask in which 10 ml of distilled water, 2ml of Ammonium Hydroxide solution and 5 ml of concentrated Amyl Alcohol was added. The mixture was made up to mark and left to react for 30minutes for colour development. The absorbance of the solution was read using spectrophometer at 505 nmλ wavelength[17].

The % phenols was calculated in the following ways:

$$\frac{100 \times AU \times C \times VF \times D}{W \times AS \times 1000 \times VA}$$

Where;

W	=	Weight of Sample Analyzed
AU	=	Absorbance of the test sample
AS	=	Absorbance of standard solution
C	=	Con. of standard in mg/ml
VA	=	Volume of Filtrate Analyzed
VF	=	Volume of Filtrate
D	=	Dilution factor where applicable

2.16.4 Determination of saponins

To determine the Saponins content of fruit bodies 2 g of the sample was dispersed in 30 ml of 7.5% ethanol. The extract was re-extracted with 100ml of ethyl acetate. The organic solvent was dried in an electric oven to a constant weight[17].

The % Saponins was calculated as

Thus:

$$\%\ \text{Saponins} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times \frac{100}{1}$$

2.16.5 Determination of tannins

To determine the tannins component of the mushroom fruit bodies, 500 mg of the powered sample was placed into a 150 ml plastic bottle and 50 ml of distilled water was added. This was shaken in a mechanical shaker for 1hour. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 3ml of 0.1 m $FeCl_3$ in 0.1N. HCl and 0.08m of Potassium Ferrocyanide. The absorbance was measured using a spectrophotometer at120 nmλ wavelength. A blank sample was prepared and the colour also developed and read at the same. A standard was prepared using tannic acid to get 100 ppm.

3 Results

The result of the micro-chemical composition of *P. ostrealits var florida* as affected by substrate and substrate combination is presented in table 1.

The result shows that the fruit-bodies of the mushroom obtained from the substrates and their combinations contained various quantities of alkaloids, flavonoids, tanins, saponins and phenols.

Table 1: Myco-chemical composition of *Pleurotus ostreatus var florida* as affected by substrate and substrate combination

Substrates	Alkaloids (g/100g)	Flavonoids(g/100g)	Tannin (g/100g)	Saponin (g/100g)	Phenol (g/100g)
D	3.73±0.031	0.18±0.026	0.38±0.006	2.66±0.006	1.04±0.03
G+D	3.84±0.025	0.24±0.006	0.46±0.006	2.447±0.025	1.16±0.01
S	3.93±0.010	0.35±0.006	0.47±0.006	2.558±0.010	1.27±0.06
S+D	3.77±0.030	0.23±0.030	0.45±0.055	2.867±0.006	1.057±0.02
Mean	3.8175	0.25	0.4392	2.63	1.133
LSD $_{0.05}$	0.037	0.031*	0.013*	0.041*	0.032*

Each value is a mean of 3 replicates ± standard deviation. Values in the column having a lower difference in value to its LSD are significantly different (P <0.05). (D= *Delonix regia*, G= *Gmelina arborea*, and S= *Saccharum officinarum*

The quantities of alkaloids obtained from the different substrate levels did not vary much. However that from *Saccharum officinarum* straw (3.93± 00g/100g) was slightly higher than the obtained from the other substrates. Flavonoids were significantly higher in fruit-bodies of *S. offianarum* than those of *Delomix regia, Gmelina arborea* and their combinations. Tannins and phenols were lowest in fruit-bodies from *D. regia,* while saponins from all the substrates did not differ significantly.

Similarly, the results of the myc-ochemical composition of the fruit – bodies of *Pleurotis pulmonarius* produced from the same substrates and substrate combination are presented in table 2.

Alkaloids from all the substrates differed only slightly. The same applied to flavonoids, tannins and saponins. However, phenols from *S. offianarum* appeared higher than those from *D. regia, G. arborea* and their combinations.

Generally, however, the flavonoids, tannins and saponins of *P. plumonarius* fruit-bodies from all the substrates appeared higher than those of *P. ostreatus* var *florida.*

The proximate composition of the fruit-bodies of *P. ostreatus* var *florida* and *P. pulmonarius* are represented in tables 3 and 4.

The results obtained, indicate that the fruit-bodies of the two mushroom species obtained from the substrates and substrate combinations contain appreciable quantities of protein ash, fats, crude fibres, and carbohydrates.

The protein content of the fruit-bodies of both *P. ostreatus* var *florida and P. pulmonariusa* from *S. offianarum* appeared lower than those from the other substrate and the combinations. In general however, the protein contents of the fruit-bodies of *P. pulmonariusa* (27.15% ±0.006 - 28.44%) appears higher than those of *P. ostreatus* var *florida* (26.66%±0.006 - 27.28±0.02%), while the carbohydrate content of fruit-bodies of *P. ostreatus* var *Florida* (36.17±0.03 – 36.89± 0.04%) appeared slightly higher than those of *P. pulmonaruis* (35.21±0.10 – 35.76±0.04%).

4 Discussions

The proximate composition of the-fruit bodies of *Pleurotus ostreatus* in table 1 and *P. pulmonarius* in table 2 grown on different substrates produced fruit-bodies with appreciable quantities of proteins, ash, fats, oil crude fibre, moisture content and carbohydrates.

The substrates produced appreciable amount of moisture, ash, fat, protein but did not show significance on the fibre content and % CHO.

Table 2: Myco-chemical composition of *Pleurotus pulmonarius* as affected by substrate and substrate combination

Substrates	Alkaloids (g/100g)	Flavonoids (g/100g)	Tannin (g/100g)	Saponin (g/100g)	Phenol (g/100g)
D	3.23±0.031	0.4233±0.006	0.57±0.058	3.0733±0.015	0.827±0.061
G+D	3.54±0.025	0.4467±0.006	0.62±0.006	3.2533±0.023	1.047±0.025
S	3.69±0.01	0.4733±0.006	0.61±0.055	3.4700±0.044	1.140±0.020
S+D	3.45±0.03	0.4533±0.02	0.58±0.066	3.1500±0.066	0.950±0.03
Mean	3.48	0.4492	0.596	3.2367	0.99
LSD $_{0.05}$	0.048***	0.017***	0.098	0.055***	0.07***

Each value is a mean of 3 replicates ± standard deviation. Values in the column having a lower difference in value to its LSD are significantly different (P <0.05). (D= *Delonix regia*, G= *Gmelina arborea*, and S= *Saccharum officinarum*

Table 3: The proximate composition of *Pleurotus ostreatus* var *florida* fruit-bodies from different substrates and substrate combinations

Substrates	MC %	Ash %	Fat %	Fibre %	Protein %	CHO %
D	4.83±0.031	10.62±0.025	2.45±0.012	17.91±0.023	27.28±0.02	36.89±0.04
G+D	5.46±0.038	10.44±0.023	2.26±0.00	18.45±0.025	26.83±0.025	36.56±0.09
S	6.13±0.012	10.25±0.023	2.16±0.023	18.61±0.029	26.66±0.025	36.17±0.03
S+D	5.16±0.030	10.33±0.035	2.38±0.023	18.25±0.042	26.96±0.025	36.91±0.10
Mean	5.395	10.41	2.31	18.31	26.93	36.63
LSD $_{0.05}$	0.051***	0.049***	0.028***	0.053	0.037***	0.059

Each value is a mean of 3 replicates ± standard deviation. Values in the column having a lower difference in value to its LSD are significantly different (P <0.05)

Table 4: The proximate composition of *Pleurotus pulmonarius* grown on different substrates and substrate combination

Substrates	MC %	Ash %	Fat %	Fibre %	Protein %	CHO %
D	6.48±0.295	11.17±0.025	2.54±0.00	16.05±0.02	28.20±0.03	35.76±0.04
G+D	6.86±0.035	10.93±0.023	2.46±0.02	16.10±0.03	28.08±0.03	35.55±0.09
S	6.933±0.042	10.85±0.023	2.51±0.02	16.27±0.05	27.15±1.72	35.21±0.10
S+D	6.420±0.035	11.23±0.031	2.64±0.02	15.89±0.04	28.44±0.03	35.34±0.03
Mean	6.673	11.0450	2.5400	16.0775	27.97	35.465
LSD $_{0.05}$	0.28**	0.048***	0.033***	0.058***	1.62	0.13***

Each value is a mean of 3 replicates ± standard deviation. Values in the column having a lower difference in value to its LSD are significantly different (P <0.05)

Protein content of the both fungi was high side with (D) *P. pulmonarius and Pleurotus ostreatus* having the highest at 28.20±0.03 and 27.28 ± 0.02 %, respectively. Fat has the lowest percentage average among other parameter at 2.31 % for *P. ostreatus* and 2.34 % for *P. pulmonarius.*

The high level of % CHO both in table 3 and 4 may not mean high level of sugar in the fungi since established that carbohydrate occurs in the mushroom in the form of Arabitol, Mannitol, Tetrahalose, and Xylose[5].

The average protein content as recorded above falls in line with involving *Agaricus bisprorus, Lentinula edodes* and *Pleurotus ostreatus* where protein content showed varying result but was average at 24%[19]. Crude protein was found mainly in *Pleurotus ostreatus* (27.23%) than in *P. djom* (24.83%)[9]. Similarly, the

protein content of four Nigeria mushrooms, *Auricularia auriculia, Pleurotus squarrosulus P. tuber – regium* and *Russula spp* to range from 15 – 24.96 g/100g on dry matter basis[10, 20].

The results of the myco-chemical composition of *Pleurotus ostreatus* and *P. pulmonarius* is shown in table 3 and 4 having alkaloids, flavonoids, tannins, saponins and phenol respectively at varying average levels and levels for the individual substrates. The high value of alkaloids among the other myco-chemicals is similar to the work published in the Journal of Pharmacy and Biological science which showed alkaloids in high values among young and matured fruit-bodies from Kaya sawdust and young and matured fruit bodies from *Adropogon* straw[21, 22]. There is a confirmation that the content of the substrates actually affects the contents of the mushrooms cultivated on them judging from the fact that protein content of fruit-bodies of both fungi was high with *Deloix regia* as substrate[23,24]. *P. pulmonarius and Pleurotus ostreatus* with 28.20±0.03 and 27.28 ± 0.02 percentage protein respectively.

5 Conclusion and Recommendations

This study focused on the Myco-chemical and Proximate evaluation of Oyster Mushrooms (*Pleurotus ostreatus* var florida*,* (mont) singer. *and Pleurotus pulmonarius* (fr) quel*)* grown on bark of *Gmelina arborea* roxb.*,* straws of *Saccharum officinarum* L. and pods of *Delonix regia* (boj. ex hook.) raf.

In view of the obtained results which had high cellulose base substrates having higher carbohydrate while Legume-based substrates have higher protein content. It is therefore recommended that famers should use legume-based substrates to boost specific nutrient targets.

6 Conflict of interests

None

7 Authors contributions

OIC and OVO, ran the experiment, gathered the data, wrote the introduction and presented the results while IGC did the analysis and proof-read the write up.

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