Australian Journal of Science and Technology

ISSN Number (2208-6404)
Volume 5; Issue 2; June 2021

Research Article

Productivity, proximate, and phytochemicals contents of *Pleurotus ostreatus* (Jacq.ex. Fr. P. Kumm.) fruit bodies produced on carbonized sawdust substrate

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ABSTRACT

Mushrooms require carbon for their growth and development more than any other mineral element. The bulk of this carbon is found in the substrates where they grow. This study was therefore, conducted to determine the influence of carbonized sawdust (CS) on the fructification and some myco-chemical constituents of *Pleurotus ostreatus* fruit bodies. A given quantity (2.5 kg) of dry sawdust was carbonized at 15, 25, and 35 min and made into five replications of 500 g each, including control of non-CS. Actively growing mycelium (50 g spawn) of *P. ostreatus* was used to inoculate each replicate substrate, stuffed in 2.5 l transparent plastic bucket. Experiment was conducted in a completely randomized design. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0, means were separated using Turkey's honestly significant difference at 5% level of significance. Results revealed that 15 and 25 min CS produced primordia within the shortest duration of 14 days. Control produced the highest (140) number of fruit bodies with smallest pileus diameter (3.78±1.06) which resulted to low fruit body yield (141.33 g) and biological efficiency (5.65%) when compared to 25 (524.83 g and 20.99%), 25 (376.89 g and 15.08%), and 35 min (513.42 g and 20.54%). Sawdust carbonization triggered a significantly ($p \leq 0.05$) decreased amount of ash from (4.28±0.03 control - 3.53±0.06 in 25 min CS); crude protein from (20.17±0.01 control - 19.50±0.13 in 15 min CS), and ether extract from (5.18±0.03 control - 3.71±0.02 in 35 min CS); but enhanced crude fiber from (3.64±0.02 in control - 4.71±0.01 in 15 min CS) and carbohydrate contents from (56.47±0.13 control - 60.35±2.26 in 35 min CS). Finally, CS significantly ($p \leq 0.05$) decreased the concentration of studied phytochemicals in the fruit bodies when compared to control. Therefore, commercial oyster mushroom growers targeting bigger sized, crude fiber, and carbohydrate rich-fruit bodies should adopt sawdust carbonization technique for more yield and profit maximization.

Keywords: Oyster mushroom, carbonization, fruit body yield, profit maximization.

Submitted: 15-05-2021, **Accepted:** 21-05-2021, **Published:** 30-06-2021

INTRODUCTION

Mushroom is a general term applied mainly for the fruiting – bodies of macro – fungi that belong to the division *Basidiomycota* and a few members of the division *Ascomycota*. They are saprophytes and are known to grow on a wide variety of substrates and habitats. However, the fact still remains that mushrooms show preference on particular substrate within a habitat.[1] The fruiting-bodies of most mushrooms are shaped like an umbrella with central stalk (stipe) supporting a cap (pileus), which bears gills (lamellae) that produce spores on the lower side. In some species of *Pleurotus*, the stipe may be absent, especially those growing on wood.[2] Mushrooms which may be epigeous or hypogenous, can be large enough to be seen with the unaided eyes and can be picked by hand.[3] There are edible and poisonous mushrooms and both categories possess nutrient and medicinal values. The ever-growing need of cheap nutritious foods and the lack of protein in developing countries have led to the development of mushroom cultivation initiative.[4]

Mushrooms are rich sources of carbohydrates, proteins, vitamins, and minerals. Mushrooms grow on decay organic matters rich in lignin, cellulose and other complicated

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carbohydrates, and on large quantities of agro-industrial wastes that are produced worldwide often causing environmental and health problems. Mushroom cultivation represents the only current economically viable biotechnology process for the conversion of waste plant residues from forests and agriculture.[5]

According to Wood and Smith,[5] mushroom cultivation represents the only current economically viable biotechnological process for the conversion of waste plant residues from manufacturing industries, forests and agricultural farmlands into protein rich food (mushrooms), and other valuable intermediate or finished products. Conversion of lignocellulose into food and feed rich in protein by fungi offers an alternative means for developing unconventional sources of proteins as food/feed.[6] Hence, the use of different agricultural wastes such as banana leaves, Alam grass (*Imperata cylindrica*) straws, bagasse, husk, pods, pulp, waste paper, and corn cobs as substrates is a good development.

Agro-wastes are ordinarily not of importance to humans. They are unsightly and constitute nuisance to the society. Agro-wastes also cause forest fires leading to the destruction of lives and properties. Mushrooms are known to grow on a wide variety of substrates and habitats.[1] Most of the edible fungi have strong enzyme system and are capable of utilizing complex organic compounds, which occur as agricultural wastes and industrial by-product. Hence, various agricultural by-products are being used as substrates for the cultivation of oyster mushrooms. The agricultural wastes are converted into edible biomass in the form of fruit-bodies.

Carbonization of substrate has been reported to be an effective means of growing mushroom.[7] Biochar derived from carbonizing substrates have been used to improve the carbon composition of mushroom and has also been used as soil amendment to improve soil productivity, carbon storage, and filtration of percolating soil water.[8] Hence, it is necessary to study and document the outcome of using carbonized sawdust (CS) as substrate for the growth and yield of *Pleurotus ostreatus* fruit bodies.

The aim of this investigation is to assess the yield of *P. ostreatus* fruit-bodies grown on carbonized saw dust to determine the effect of the carbonized saw dust on the micro-chemical contents of the *P. ostreatus* fruit-bodies and to determine the influence of CS on the fruiting duration of *P. ostreatus* fruit-bodies. It is to evaluate the effect of carbonization on some macro-morphological characteristics of *P. ostreatus* fruit-bodies, determine the influence of CS on the yield and biological efficiency (B.E) of *P. ostreatus* fruit-bodies and to ascertain the effect of carbonization on some myco-chemical composition of *P. ostreatus* fruit-bodies.

Many researches have been carried out to evaluate the use of different substrates in the cultivation of oyster mushroom especially *Pleurotus ostreaus*. Baysal *et al*.[9] conducted an experiment involving the cultivation of *P. ostreatus* on waste paper with addition of chicken manure, peat, and rice husks. They reported that increasing the amount of rice husks in the substrate accelerated spawn run duration, Pinhead and fruiting body formation. Iloka[10] reported that the best substrate for cultivation are corn cob and rice husk; because analyses showed that these substrates were identified to be rich in cellulose which are vital for growth and fruiting of oyster mushroom. Nwoko *et al*.[11] cultivated *Pleurotus pulmonarius* (Fries) Quel. using tree logs of *Dacryodes edulis*, *Mangifera indica, and Treculia Africana.* They reported that *P. pulmonarius* fruit bodies harvested from various tree logs were significantly different $P < 0.05$ in their nutritional and bioactive compounds composition and that the fruit body samples were rich in protein, carbohydrates, Na, K, and Ca. Nwokoye *et al*.[12] also observed that fruit bodies contained significant amount of alkaloids, tannins, and saponins[12] studied the carbon and nitrogen requirements for mycelial growth of *P. ostreatus.* In that study, three carbon sources where used, namely glucose, maltose, and starch. It was observed that glucose was the best carbon source for *P. ostreatus* followed by maltose and starch. The preference of glucose over other carbon source by *P. ostreatus* may be due to the ease with which it was metabolized to produce cellular energy for the growth of the organism. Kurtzman and Zadrazil[13] mentioned that the ability of *Pleurotus* spp to use different carbon sources may be an expression of the physiological differences in the species or of the isolates, since other isolates of the same species might give different results. Anyakorah *et al*.,[14] in a similar investigation, used six carbon sources, ribose, starch, dextrin, glucose, mannose, and cellulose. It was found that the carbon compounds supported growth except ribose, starch, and dextrin. Cellulose was the most utilized carbon followed by glucose and mannose. Fruiting duration of *Pleurotus sajor-caju* was assessed by Pokhrel *et al*.[15] on three different substrates, namely, maize stalk, pea residue (tendrils), and banana leaves with and without supplementation of rice bran and chicken manure. Faster fruiting duration was obtained from maize stalk with rice bran and second-best was recorded from pea residue with rice bran. Among the substrates used, maize stalk appeared best followed by pea residue and banana leaves.[15]

In a study conducted by Ogbodo,[16] rice hull charcoal application resulted in a large increase in soil nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and superior growth of plants. Higher soil organic carbon (OC) and available potassium (K) and increased below ground biomass production of the plant amended with rice hull charcoal was also reported by Abrishamkesh *et al*.[17] A similar improvement in the availability of plant nutrients with rice hull charcoal application was also obtained in the study of[18,19] investigated the growth and yield parameters of three strains

of *Pleurotus eryngii* such as Pe-1 (native to Bangladesh), Pe-2 (germplasm collected from China), and Pe-3 (germplasm collected from Japan) cultivated on sawdust and rice straw. Pe-1 on sawdust showed the highest biological yield and BE (73.5 %) than other strains. Furthermore, the mycelium run rate and number of fruiting bodies were higher in Pe-1 than other two strains. The quality of mushroom strains was near about similar. On sawdust, the yield and efficiency were better than those cultivated on rice straw; however, on straw the mushroom fruiting bodies were larger in size.

Pleurotus florida cultivation on different substrate compositions has been investigated by Mondal *et al*.[20] Highest biological yield and economic yield (164.4 g and 151.1 g) was obtained from rice straw which was much higher than control. A number of papers devoted to the study of the process of *P. pulmonarius* cultivation on different mixtures of cotton waste and cassava peel,[21] cotton waste alone, and combined with rice, coir fiber, oil palm waste, sawdust of Gmelina arborea, and rice straw. Mycelial growth, colonization period, primordial initiation, harvesting time, yield, mushroom size, and BE of *P. sajor-caju* were assessed on three different substrates namely maize stalk, pea residue (tendrils) and banana leaves with and without supplementation of rice bran and chicken manure. The highest yield (348.13 g) with 87.03 % BE was obtained from maize stalk with rice bran and second-best yield (299.53 g) with 74.88 % BE was recorded from pea residue with rice bran. Among the substrates used, maize stalk appeared best followed by pea residue and banana leaves. Rice bran showed best supplementation for mycelial growth and yield with all substrates.[15]

On the influence of rice husk in the B.E and nutrient content of *P. ostreatus*,[22] evaluated rice husk as a possible additive to composted saw dust of *Triplochiton sclerexylon*, to ascertain its contribution to the B.E and nutrient content of *P. ostreatus.* They found that the mean mycelia growth rate and the spawn run period on the substrate showed an increasing trend in B.E with increased rice husk concentration. Due to the fact that rice husk dries up very fast, it is advisable to use it as an additive to saw dust rather than using it alone at 100% concentration.[23]

Atopia *et al.*[24] observed that *Pleurotus* can also be cultivated on cellulosic residues from rice straw to improve the B.E and nutrient content. All their treatments showed significant differences in biological efficiencies at $P \leq 0.05$. Based on the increase in the B.E of *P. ostreatus,* they concluded that rice straw and rice husk at 1:1 ratio can be used as an alternate substrate for producing more mushroom in rice growing areas. Mushrooms contain appreciable quantities of crude fibers, although little information exists on the total dietary fiber contents of mushrooms. Okwulehie *et al*.[25] reported high crude protein (CP) and carbohydrate contents in *P. ostreatus* var florida fruit bodies cultivated on different substrates and substrate supplementations.

According to Okwulehie and Odunze,[26] mushrooms generally contain low-oil and fat, and because of the low content of oil and fat in mushrooms, they are recommended as good supplements for patients with cardiac problems.

The proximate, macro-element, and mineral contents of *P. ostreatus* var *florida* fruit bodies grown on different substrates and substrate supplementations were carried out by, Okwulehie *et al*.[25] The findings showed significant high levels of protein, carbohydrate, vitamins, fats, and oil in the substrates studied. They also evaluated the influence of two levels of organic manure on macro-element and vitamin contents of *P. ostreatus* to understand the best ways to use these manures for sustainable cultivation of the macro-fungus. The proximate, macro element, and vitamin composition of the fruit bodies of *P. ostreatus* as affected by substrate and additive types were in appreciable quantities.

In another experiment, Okwulehie *et al*.[27] also studied the myco-chemical, proximate mineral and vitamin composition of two mushrooms; *Pleurotus squarrosulus* and *Termitomyces robustus.* The result of the investigation showed that both mushrooms have high nutritional potentials. In their study to determine the influence of different substrates on the nutritional composition of *P. pulmonarius*, Okwulehie *et al*.[28] reported that substrate type influenced the bioactive nutrients and vitamin constituents of oyster mushrooms. The productivity and proximate content of *Pleurotus sajor-caju* studied by Patil[29] revealed that using different agro-wastes such as Soybean, paddy straw, wheat or dowar straws, sunflower, and Pigeon pea stalks, Soybean straws showed the highest yield with 83.00% B.E, maximum protein (25.80%), fat 92.82%), and ash (7.30%) content. He also reported that the variation of these nutrients content might be due to the quality and quantity of nutrients available to substrates.

Okwulehie *et al*.,[30] reported that the myco-chemical composition of matured fruited-bodies of the mushroom are affected by substrates and substrates supplementation. They reported that the mushrooms produced from substrates *Andropogon gayanus* + *Anthonata. macrophylla* bark mixed at 80% + 20% and 50% + 50%, contained little quantities of tannins, HCN, and anthocyanin which means that the mushrooms are safe for consumption. The flavonoids contents of the mushroom indicate their medicinal value, meaning that the consumption of the mushroom may prevent oxidative cell damage and have strong anticancer activity.[31]

MATERIALS AND METHODS

Pure mycelia culture of *P. ostreatus* was obtained from the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia state.

The sawdust used for this was obtained from timber main market located along Ikot-Ekpene road Umuahia, Abia state.

The study was conducted at the mushroom house of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located between longitude 7^0 and 70^005^0E and latitude 5^0 and 5^025^0N; with humid tropical climate. Rainfall is bi-modally distributed with peaks between July and September of each year. Annual rainfall is approximately 170 mm, spread between April and November each year.[32] Experiment was conducted in completely randomized design. The sawdust substrate was divided into four portions of which the first portion was carbonized for 15 min, the second portion was carbonized for 25 min, the third portion was carbonized for 35 min, and the last portion was used as the control with no carbonization. Each experiment was made into four replications each.

Substrate Carbonization/Preparation

Sawdust measuring 2500 g each was carbonized separately in a heated metallic drum at 15, 25, and 35 min at 100°C. Another sawdust measuring 2500 g served as control. During heating, the substrate was stirred regularly using a wooden stirrer to ensure uniform circulation of heat. The three levels of CS were allowed to cool, after which they were moistened with tap water according to the modified method of.[3] Palm test method was used to determine optimum moisture content of the sawdust.

Preparation of Cropping Room

Before the substrate inoculation, the cropping room floor was scrubbed with water and detergent. The walls were got rid of cobwebs and disinfected with Detol disinfectant. The room was well illuminated and ventilated by opening the window panes. This equally maintained the daytime temperature at 27±2°C and improves ventilation in the room.[26]

Substrate pasteurization

All the substrates were separately stuffed into "Bagco" bags, codified, and lowered into metallic pasteurization column and were pasteurized for 3 h at 100°C. After that, they were allowed to cool overnight.

Substrates Inoculation

The substrate and their combination dispensed in 2–5 l perforated transparent plastic buckets perforated were inoculated following the method of Okwulehie and Okwujako,[33] actively growing spawn of *P. ostreatus* was sprayed in layers of the substrate.

Fruiting and Harvesting

Fruiting of the mushroom started on the different carbonized substrates from the 14th day after inoculation. The first flush was harvested from the September 25–30, 2019, and the second flush was harvested from the November 2–7, 2019.

Parameters Measured

I. Yield/Number of fruit-bodies
   The fruit bodies were counted directly after splitting them from their bunch from each bucket.
II. Pileus diameter (PD) (cm)
   This was measured in centimeter (cm) with a transparent plastic ruler from one edge of the pileus across the stipe to the other edge.
III. Stipe length (SL) (cm)
   Similarly, the length of the stipe was also measured in centimeter (cm) using a transparent plastic ruler.
IV. Fresh and dry fruit-bodies
   The fresh and dry weight of the fruit-bodies were obtained by weighing the fruit bodies using a digital scale (Model, 2000) made in USA.

The drying of fresh fruit-bodies was done using a hot air oven, at 80°C for 48 h.

Determination of B.E: The BE, that is, the percentage yield of fresh mushroom fruit bodies per dry weight (g/kg) substrate was calculated using the formula recommended by,[8] namely:

$$\text{B.E} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times \frac{100}{1}$$

Determination of the Phytochemical Composition

I. Flavonoids

Flavonoids in the dry samples was determined following the methods of by Chang and Miles,[3] 10g of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The solution was filtered through Whatman 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 even to a constant weight.

The flavonoid content was expressed in percentage thus:

$$\% \text{ Flavonoids} = \frac{= \text{weight of residue}}{\text{weight of sample}} \times \frac{100}{1}$$

II. Alkaloids

To estimate the quantity of alkaloids, 5 g of the dry powdered sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated by evaporating in boiling water to one quarter of the original volume. Concentrated Ammonium hydroxide (NH_4OH) 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 by Harborne, 1973.[34] The alkaloids were expressed in percentage as:

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

III. Tannins

Tannins were determined according to the method of (Harborne, 1973).[34] A measure (0.5 g) of the sample was dispensed into a flask containing 10 ml of 2 m HCl and shaken for 5 min and transferred into a volumetric flask and made up to 50 ml. The mixture was filtered and 5 ml of the filtrate was introduced into a test tube. 3 ml of 0.1 NHCl and 3 ml of 0.008 m of potassium ferro-cynide ($K_3F[CN]_3$) were added. The absorbance was read at 720 nm within 10 min.

IV. Saponins

A measure of 0.1 g of the powdered samples were boiled with 5 ml of distilled water for 5 min and decanted while still hot. The filtrate was used for frothing and emulsion tests.

Frothing test: One milliliter of the filtrate was diluted with 4 ml of distilled water and the mixture shaken vigorously and observed on standing for suitable froth.

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

V. Phenols

The phenolics content of the sample (fat-free sample), 2 g of the sample was defatted with 100 ml of diethyl ether, using Soxhlet apparatus for 2 h. To extract the phenols component of the sample, the fat-free sample was boiled with 50 ml of either for 15 min. 5 ml of the extract was Pipette into a 50 ml flask into which 10 ml of distilled water, 2 ml of ammonium hydroxide (NH_4OH) solution, and 5 ml of concentrated amyl alcohol had been added.

The mixture was made up to mark and left to react for 30 min for color development. The absorbance of solution was read using a spectrophotometer at 505 nm wave length.[35]

Statistical analysis

Data collected were analyzed by Analysis of Variance (ANOVA) as described by Steel and Torie[35] Turkey's Honestly Significant Difference test at probability level of 5% was used to separate the means and standard deviation (means ±SD).

RESULTS AND DISCUSSION

The result of fruiting body and fruiting duration of *P. ostreatus* using 15 min CS, 25 min CS, 35 min CS, and Control is presented in Table 1. The results showed that control started producing fruit bodies after 17 days with a total of 140 fruit bodies 15- and 24-min CS produced fruit body primordia after 14 days with a total of 103 and 122 fruit bodies, respectively, while 35 min CS produced fruit body primordia, after 19 days with a total of 138 fruit bodies.

The results of the morphological characters of *P. ostreatus* fruit bodies using 15 min CS, 25 min CS, 35 min CS, and control are presented in Table 2. The results showed that PD of fruit bodies harvested from 35 min CS was highest at 5.48±1.82, followed by those of 15 min CS (5.33±3.06). Fruit bodies with the smallest PD were obtained at control (3.78±1.06), followed by 25 min CS (4.03±2.18). SL was significantly ($P > 0.05$) high in fruit bodies obtained from 35 min CS at 3.46±1.63, followed by 15 min CS (2.82±0.81) while control and 25 min CS were 2.25±0.55 and 2.37±0.78, respectively. It was also observed that fruit bodies from 35 min CS had the highest weight (4.90±0.00) while the lowest was obtained at 25 min CS at 3.10±0.00.

The results of yield and B.E of *P. ostreatus* fruit bodies using 15 min CS, 25 min CS, 35 min CS, and control are presented in Figure 1. The result showed that 2500 g of sawdust carbonized at 3-time interval levels; including control produced varying amount of *P. ostreatus* fruit bodies as follows. Control produced the lowest quantity of fruit bodies at 141.33 g and 5.65% representing yield and B.E, respectively. This was followed by 25 min at yield (376.89) and B.E of 15.08%. 35 min CS produced fruit body yield of 513.42 g and BE of 20.54% while 15 min produced the highest amount of fruit bodies at yield of 524.83 g and BE of 20.99%.

The result of proximate composition of fruit bodies of *P. ostreatus* using 15 min CS, 25 min CS, 35 min CS, and

Table 1: Fruiting duration and fruit body number of *Pleurotus ostreatus*

Substrate	Fruiting duration	FBN
Control	17	140
15 min CS	14	103
25 min CS	14	122
35 min CS	19	138
Mean	16	125.75

FBN: Fruit body number, CS: Carbonized sawdust

Table 2: Morphological characters of *Pleurotus ostreatus* fruit bodies

Substrate	PD (cm)	S.L (cm)	WT (g)
Control	3.78±1.06^a	2.25±0.55^a	3.70±0.00^b
15 min CS	5.48±1.82^b	3.46±1.63^c	4.90±0.00^d
25 min CS	4.03±2.18^a	2.37±0.78^a	3.10±0.00^a
35 min CS	5.33±3.06^b	2.82±0.81^b	3.80±0.00^c

Means followed by the same alphabet within column are not significantly different by Turkey's HSD test ($P \leq 0.05$), means±SD ($n=3$). PD: Pileus diameter, S.L: Stipe length= weight, CS: Carbonized sawdust

control is presented in Table 3. The results showed that control produced fruit bodies with the highest MC of 10.27±0.04 while 15, 25, and 35 min CS were recorded at 9.85±0.01, 10.05±0.04, and 10.17±0.17, respectively. Dry matter content of fruit bodies was highest (90.15±0.01) and lowest in the control (89.73±0.04). Fruit bodies from control had the highest ash content (4.28±0.03), compared to other treatment levels. A similar result was observed in the CP and ether extract (EE) of fruit bodies. CP content of fruit bodies from 15, 25, and 35 min CS and EE contents of fruit bodies from 15- and 25-min CS as well as CHO contents of fruit bodies from all levels of treatment was not significant ($P > 0.05$).

The result of phytochemical composition of fruit bodies of *P. ostreatus* using 15 min CS, 25 min CS, 35 min CS, and control is presented in Table 4. The results of phytochemical composition of fruit bodies indicated that flavonoids content of fruit bodies from control was recorded at 1.81±0.01 while those of 15; 25-; and 35-min CS were found at 1.73±0.01, 1.76±0.00, and 1.76±0.00, respectively. Alkaloids were found at 0.58±0.02 in fruit bodies from control but 0.49±0.01, 0.44±0.02, and 0.37±0.01, respectively. Tannins content was recorded at 1.87±0.04, 1.74±0.02, 1.79±0.01, and 1.82±0.03 in fruit bodies from control 15, 25-, and 35-min CS, respectively. Highest amount of saponins was found

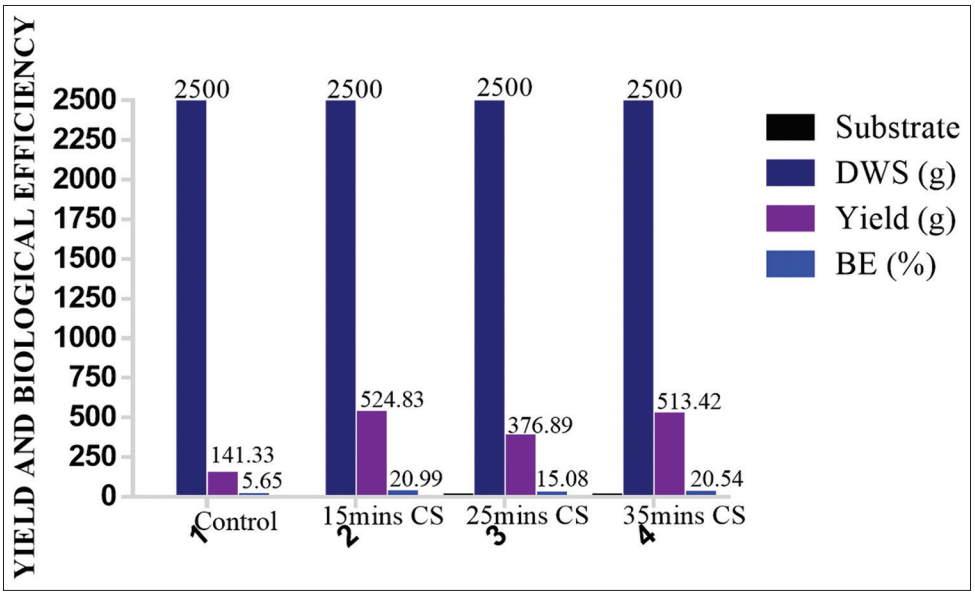

Figure 1: Yield and biological efficiency of *Pleurotus ostreatus* fruit bodies. DWS: Dry weight substrate, BE: Biological efficiency, CS: Carbonized sawdust

Table 3: Proximate composition of fruit bodies

Substrate	MC	DM	ASH	CP	EE	CF	CHO
Control	10.27±0.04^{c}	89.73±0.04^{a}	4.28±0.03^{c}	20.17±0.01^{b}	5.18±0.03^{c}	3.64±0.02^{a}	56.47±0.13^{a}
15 min CS	9.85±0.01^{a}	90.15±0.01^{c}	3.77±0.04^{b}	19.50±0.13^{a}	4.32±0.12^{b}	4.71±0.01^{d}	57.85±0.21^{a}
25 min CS	10.05±0.04^{b}	89.84±0.05^{b}	3.53±0.06^{a}	19.70±0.09^{a}	4.08±0.06^{b}	4.21±0.04^{c}	58.43±0.01^{a}
35 min CS	10.17±0.17b,^{c}	89.84±0.05^{b,c}	3.67±0.02^{b,c}	19.79±0.01^{a}	3.71±0.02^{a}	3.83±0.04^{b}	60.35±2.26^{a}

Means followed by the same alphabet within column are not significantly different by Turkey's HSD test ($P \leq 0.05$), means± SD ($n=3$). MC: Moisture content, DM: Dry mater, CP: Crude protein, EE: Ether extract, CF: Crude fiber, CHO: Carbohydrate, CS: Carbonized sawdust

Table 4: Phytochemical composition of fruit bodies

Substrate	FLV.	ALK.	TAN.	SAP.	PHEN.
Control	1.81±0.01^{c}	0.58±0.02^{c}	1.87±0.04^{b}	0.85±0.00^{c}	0.44±0.02^{b}
15 min CS	1.73 ±0.0^{a,b}	0.49±0.01^{b}	1.74±0.02^{a}	0.81±0.02^{b,c}	0.34±0.03^{b,c}
25 min CS	1.70±0.00^{a}	0.44±0.02^{a,b}	1.79±0.01^{a,b}	0.76±0.01^{a,b}	0.31±0.01^{a}
35 min CS	1.76±0.00^{a,b}	0.37±0.01^{a}	1.82±0.03^{a,b}	0.73±0.01^{a}	0.23±0.01^{a}

Means followed by the same alphabet within column are not significantly different by Turkey's HSD test ($P \leq 0.05$), means ± SD ($n=3$). FLV.: Flavonoids, ALK.: Alkaloids, TAN.: Tannins, SAP.: Saponins, PHEN.: Phenols, CS: Carbonized sawdust

in control (0.85±0.00) and 15 min CS (0.81±0.02) while lowest was recorded in fruit bodies from 25 (0.76±0.01) and 35 min CS (0.73±0.01). Phenols content was high in fruit bodies from control (0.44±0.02) compared to those of 15, 25-, and 35-min CS which were not significantly different at 0.05 probabilities.

DISCUSSION

Results of the investigation revealed that control started producing fruit bodies after 17 days with a total of 140 fruit bodies. It was also observed that 15- and 25-min CS produced fruit body primordia after 14 days with a total of 103 and 122 fruit bodies, respectively, while 35 min CS produced fruit body primordia, after 19 days with a total of 138 fruit bodies. Moonmoon *et al*.[19] recorded longer fruiting time of *P. ostreatus* with saw dust, wheat straws, and other agro-wastes combination. They reported that the longest fruiting duration was 35 days while the shortest was 27 days. The short period of commencement of fruiting recorded in this work especially at 15- and 25-min CS, could be due to the fact carbonizing sawdust within this time frame could provide the optimum biotic and abiotic environment required to trigger early fruiting, possibly by lowering the lignocellulosic composition of the sawdust.

The period of 15 and 25 min CS produced the smallest number of fruit bodies at 103 and 122. This justifies the claims by Okwulehie *et al*.[25] who noted that substrates with low lignocellulosic content produce fruit bodies earlier and for a shorter time compared to those with high lignocellulosic content which produce fruit bodies later but fruit for a longer time.

Results of morphological characters of fruit bodies indicated that PD of fruit bodies harvested from 35 min CS was highest at 5.48±1.82, followed by those of 15 min CS (5.33±3.06). The mean PD of fruit bodies from all levels of CS substrates; including control was higher than the values reported by Okwulehie *et al*.[25] with uncarbonized substrates. The large PD obtained in this study could be due to high amount of carbon in the CS substrate. According to Onyeizu *et al*.,[32] large mushroom cap diameter is a desirable market quality and growers must ensure the production quality fruit bodies to maximize profit. SL was significantly ($P > 0.05$) high in fruit bodies obtained from 35 min CS at 3.46±1.63, followed by 15 min CS (2.82±0.81) while control and 25 min CS were 2.25±0.55 and 2.37±0.78, respectively. A similar result was obtained on SL and fruit body weight and could also be attributed to high carbon content of fruit bodies. Therefore, carbonizing sawdust at different time intervals could greatly influence fruit body morphology, especially in oyster mushrooms as reported by Rollon *et al*.[18] Results showed that 15 min CS produced the highest number of fruit bodies at yield of 524.83 g and BE of 20.99%. This shows that heating sawdust for 15 min provides optimum carbon requirement for yield of oyster mushroom. Okwulehie *et al*.,[25] however, reported that improved growth and high yield obtained from carbonized corncob were due to higher nitrogen content and a lowered C/N ratio. In general, high fruit body yield was recorded at all levels of CS, except in control; when compared the overall yield and B.E of *P. ostreatus* fruit bodies as observed in this experiment were significantly higher than those obtained by Okwulehie *et al*.[28] who cultivated *P. ostreatus* on sawdust amended with different agro-wastes,[11] who grew *P. ostreatus* on trees logs,[33,36] who in their separate investigations cultivated oyster mushrooms on different agro-waste components. On the other hand, this experiment recorded low B.E. Rip[37] reported that B.E of 100% and above is a comparatively favorable fruit body yield and mostly obtained by experienced commercial mushroom growers.

Results showed significant difference ($P \leq 0.05$) in all studied parameters except carbohydrate. It was also observed that carbonization of sawdust at 15, 25, and 35 min significantly reduced Ash, CP, and EE contents of their respective fruit bodies.

Values obtained in all the studied parameters (MC, Ash, EE, CF, protein, and CHO) were relative to those obtained by various researchers such as Okwulehie and Okwujako[33], Patil *et al*.[38] and Okoi and Iboh.[36] Results further reveals that CHO composition of fruit bodies increased from 56.47±0.13 in control to 60.35±2.26 in 35 min CS. Chang and Miles[3] maintained that high CHO contents of mushrooms is due to the high carbon and lingo-cellulosic compositions in the substrate where they grow; which mushrooms were able to break down using extra cellular enzymes. The appreciable amounts of vital nutrients in *P. ostreatus* fruit bodies as generally observed in this study have been attributed to the nature of substrate and to a large extent, mushroom species[11] This further substantiates the claims by Obodai *et al*.,[39] Adejoye and Fasidi[40] and Okoi and Iboh[36] which in separate experiments noted that the nutritional composition of mushrooms could reflect the chemical composition of the substrate used, as mushrooms are able to carry out extra-cellular digestion of the decomposed substrate during cultivation.

Results of phytochemicals composition of fruit bodies indicated that flavonoids content of fruit bodies from control was recorded at 1.81±0.01 while those of 15; 25-; and 35-min CS were found at 1.73±0.01, 1.76±0.00, and 1.76±0.00, respectively. The observed slight reduction in flavonoids concentration was probably due to heat of carbonization which may lead to loss of some volatile bioactive compounds in the substrates. Flavonoids act as anti-carcinogens, anti-bacterial.[41] A similar result was recorded in Alkaloids, tannins, saponins,

and phenols. For instance, alkaloids were found at 0.58±0.02 in fruit bodies from control but 0.49±0.01, 0.44±0.02, and 0.37±0.01, respectively. Alkaloids have powerful effect in animal physiology and are important in pharmaceutical industries, for drug manufacturing.[42] Edeoga and Erieta[42] also recorded that alkaloids are stimulants and acts by prolonging the action of several hormones. Tannins content was recorded at 1.87±0.04, 1.74±0.02, 1.79±0.01, and 1.82±0.03 in fruit bodies from control 15, 25, and 35 min CS, respectively. Highest amount of saponins was found in control (0.85±0.00) and 15 min CS (0.81±0.02) while lowest was recorded in fruit bodies from 25 (0.76±0.01) and 35 min CS (0.73±0.01). saponins are implicated in the prevention of parasitic fungal diseases[42] while tannins have been used as antitumor agents and perform a wide range of anti-infective actions[43] Phenols content was high in fruit bodies from control (0.44±0.02) compared to those of 15 min, 25 min, and 35 min CS which were not significantly different at 0.05 probability level. Although, there seems to be reduction in the concentrations of flavonoids, saponins, phenols, tannins, and alkaloids in all fruit body samples obtained from various levels of CS, when compared to control; photochemical values recorded in this experiment were higher than those reported by Okwulehie *et al*.[28]

CONCLUSION

In this experiment, it was observed that control produced fruit bodies at the 17 days, after inoculation (DAI) and had the highest number of fruit bodies with smallest sizes which gave rise to reduction in yield when compared to other levels of carbonized substrate. The shortest fruiting duration was observed in 15 and 25 min CS at 14 DAI.

Fruit body yield and B.E were highest at 15 min CS, followed by 35.

Substrate carbonization caused a slight decrease in the amount of ash, CP, and EE but increased but enhanced crude fiber and carbohydrate contents.

Finally, substrate carbonization significantly ($P \leq 0.05$) decreased the concentration of all the studied phytochemicals in the fruit bodies as they were found to be higher in control.

It is pertinent to recommend therefore that mushroom growers should carbonize their sawdust substrate between 15 and 25 min at 100°C for early primordial formation.

Commercial mushroom growers targeting bigger sized and attractive *P. ostreatus* fruit bodies should adopt substrate carbonization, especially at the threshold of 15 min. Production of crude fiber and carbohydrate-rich *P. ostreatus* fruit bodies should be increased by use of CS, but should not be encouraged in the production of protein-rich mushrooms. Adoption of CS in the production of phytochemicals–rich *P. ostreatus* fruit bodies should not be encouraged as most volatile phytochemicals are likely lost through carbonization.

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