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NUTRIENT COMPOSITION OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*), GROWN ON RUBBER WOOD SAWDUST IN CALABAR, NIGERIA, AND THE NUTRIENT VARIABILITY BETWEEN HARVEST TIMES.

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ABSTRACT: *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 Nigeria until recently. The aim of this study was to cultivate Oyster mushrooms on rubber wood sawdust as base substrate, and to evaluate the nutrient composition in the first harvest (15th day) and final harvest (34thday). At harvests, phytochemicals including, phenolics, saponins, flavonoids and alkaloids, and antinutrients including, tannates, cyanates, oxalates and phytates were detected in the mushrooms. The proximate nutrient composition gave 44.64±0.61mg/100g of total carbohydrate content and a protein content of 21.71±1.09mg/100g. Fat (4.52±0.15mg/100g) and fibre (11.42±1.05mg/100g) were quantified. These values were not significantly different between the 1st and 2nd harvests. The Energy value was 308.08±5.44 Kcal/100g and 316.01±4.75 Kcal/100g for the 1st and 2nd harvests respectively. Vitamins detected included, Vitamin A (81.22±3.51 IU); Vitamin C (27.88±0.05mg/100g); Vitamin D (2.92 ± 0.25mg/100g); Vitamin E (24.61 ± 0.60 mg/100g); Pantothenic acid (89.09±5.72mg/100g); Niacin (27.01±3.75mg/100g) and significant ($p<0.05$) concentrations of Vitamins B_1, B_2, B_6 and Folic acid. Mineral elements detected in the Oyster Mushroom included, Fe (56.44±2.7mg/100g); Zn (3.44±0.95mg/100g); Se (5.0±0.02µg/100g); Mn (1.55±0.42mg/100g); P (922.05±10.12mg/100g); Mg (15.45±1.41mg/100g) and Cu (0.71±0.22mg/100g). The core electrolyte concentrations detected include, Na (13.21±1.22 mg/100g); K (1085.09±24.08 mg/100g); Ca (32.17±3.77 mg/100g) and chloride (17.44±3.25 mg/100g). The nutrient composition of the mushroom confirms the claims that Oyster mushroom may be classified as a functional food, because it provides health benefits to all ages. We conclude that rubber wood saw dust proved an excellent base substrate for growing Oyster Mushroom in commercial scale, and the quality of Oyster mushroom harvested met national regulatory standards, and international quality standards (EU specified limits) especially with respect to heavy metals contamination, and that CRC should commercialize their product outside the borders of Nigeria.*

KEYWORDS: pleurotus ostreatus, oyster mushroom, nutrients, phytochemicals, health benefits.

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INTRODUCTION

For centuries, many traditional diets have been prepared in Nigerians 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 food supplement for centuries, not only for their flavour, aroma and nutritive values bur also for their medicinal properties (Patel et al, 2012; Deepalakshmi and 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).

Oyster mushrooms are cultivated in all climatic conditions of the world. It has become part of daily diets in the UK, China, Japan, India, European and Asian countries, and many other parts of the world, because of their high nutritional values. Oyster mushrooms like other mushrooms have been included in the category of functional foods. Functional foods are those enriched or modified and consumed as normal diet to provide health giving benefits (Rajarathanam et el.1992; Cohen et al 2002).

There are over 2000 species of mushrooms that are edible. A few of them are known to be poisonous, while over a dozen species including Oyster mushrooms are edible and very nutritious, and can be cultivated in commercial quantities for their nutritive and medicinal values (Zadrazil, 1978). The *Pleurotus* species belong to the phylum *Basidiomycota* because they produce oyster-shaped mushrooms (basidiocarp), as a result of which they have been named Oyster Mushrooms (Chang, 2006; Zadrazil, 1978). There is also, another report which claimed that, the genus *Pleurotus* comprises about 40 different species that are commonly referred to as "Oyster Mushroom" (Deepalakshmi and Mirunalini, 2014).

Oyster mushrooms has been reported to be cultivated and grown on wheat straw, grass, wood shavings, sawdust, compost waste and other organic nutrients. *Pleurotus ostreatus*, the pearl oyster mushroom or tree oyster mushroom, was first cultivated in Germany as a subsistence measure during World War 1, and is now grown commercially around the world for food (Eger et.al. 1976).

A typical oyster mushroom has a broad fan or oyster-shaped cap spanning 5-25cm and short stalk. Natural specimens colouration range from white to gray or tan to dark-brown. The spore print of the mushroom is white to lilac-gray (Eger et.al. 1976). Several reports of the nutritional and medicinal properties of *Pleurotus* species have been documented (Gregori et al. 2007; Khan and Tania, 2012; Patel et al 2012; Deepalakshmi and Mirunalini, 2014). As functional foods,

Oyster mushrooms elicit their positive effect on human beings in several ways. As earlier stated, functional food comprises products of microbial, plants and animal origin containing physiologically active compounds which benefit human health and reduces the risk of chronic diseases (Hasler, 1996; Cheung, 2008). It includes dietary supplements, nutriceuticals, medicinal foods, vitality foods, pharma foods, phytochemicals and mycochemicals foods (Hasler, 1996).
It has also been reported that many mushrooms play very beneficial role in human ailments because they possess many typical pharmacological features including; acting as metabolic activators, preventing and controlling intoxication, and microbial and viral infections, helping in immune-balancing and immuno-modulation, and as antioxidants with rejuvenating and energy boosting properties (Wasser, 2002).

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 concentration of Fe, and enhances RBC count (Tzianabos, 2000). Research have 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 (Asfors and Ley,.1993). 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) hyper-inflammatory conditions (Patel et al. 2012).

Consumption of Oyster mushrooms is said to elicit anti-cancer (Wasser, 2002), anti-viral and anti-microbial benefits in humans. Its anti-viral properties are associated with the presence of a protein known as ubiquitin, which inhibits viral replication in humans (Thompson and Moland, 2000; Wolf et al, 2008). On the other hand some polysaccharides found in Oyster mushrooms have been reported to exhibit potent anti-tumour effects *in vitro* and *in vivo* in several laboratory studies ((Wasser, 2002). The polysaccharide (pleuran), is a specific anti-oxidant shown to elicit potent benefits against colon cancer (Bobek and Galbavy, 2001).

Oyster mushrooms have also been reported to lower blood pressure and lower blood sugar concentration (Windholz, 1983; Miyazawa et.al. 2008). The lowering of blood sugar has been attributed to the presence of low fat content, high protein content and high fibre content in Oyster mushrooms. Guanide concentration in Oyster mushrooms has also been reported to exert sugar lowering effect on types 1 and 2 diabetic patients. Guanide from *Pleurotus ostreatus* is similar to the anti-diabetic drug bi-guanide (Windholz, 1983; Miyazawa et.al. 2008). Diets containing fresh fruiting bodies of Oyster mushrooms or the dried and powdered material were reported to significantly lower plasma cholesterol and lipids in humans (Bobek et. al. 1991; Hossain et. al. 2003).

Apart from its nutritional and medicinal applications, and its use as a functional food, *Pleurotus ostreatus* has also been applied in many other biopotentialities including, recycling of agricultural residues, bioremediation of polluted sites and general environmental management (Rajarathanam et al. 1992; Rai, 2007), bioconversion of lignocellulosic wastes (Sharma et al. 1996; Salmones et al. 2005), production and improved animal feed (Akinfemi et al. 2010), bioremediation and degradation of xenobiotics (Morgan et al. 1991; Buswell, 2001), Bioremediation and degradation of industrial dyes (Shin et al. 1997; Espindola et al. 2007), bioremediation of hydrocarbon wastes (Rajarathanam and Bano, 1989; Rajarathanam et al. 1992), degradation of xenobiotics for bioremediation (Masaphy et al. 1996; Reddy and Mathew, 2001), and utilization in enzyme production (Naraian et al. 2010; Daba et al. 2011). Oyster mushrooms can also be used industrially for mycoremediation purposes (Rajarathanam et al. 1992).

MATERIALS AND METHODS

The spores of the Oyster Mushrooms were purchased from China by Calabar Rubber Company (CRC). Sawdust was obtained from the Wood Processing Unit of the Calabar Rubber Company. Spawn, 6kg plastic buckets, 1kg black polythene bags, hand gloves, thermometers, laboratory glassware and other mushrooms cultivation accessories were provided by CRC. Cultivation conditions were provided by the company and were under very clean and well ventilated environment, and air conditioning.

Method of Cultivation

The study was carried out in collaboration with Calabar Rubber Company limited, and in accordance with the Oyster Mushroom growing methodology already established by CRC, with a modification of optimizing cultivation parameters (Tesfaw et al. 2015). Two rooms were cleaned and kept sterilized from a previous fumigation, with its air conditioning maintained at 18.0^{o}C. One was kept dark and away from direct sunlight. The second room was with direct sunlight and well ventilated with its air conditioning set at 23±2.00^{o}C.

To prepare the spawn, 5kg of rubber sawdust was weighed into a sterile stainless steel pot and 3 liters of water added and boiled for 30 minutes at 65-75^{o}C. It was then cooled and then transferred into 6kg plastic bucket and 1 kg of water was further added to completely hydrate it and mixed thoroughly to make it wet to form the mushroom growth nutrient medium. The mushroom medium was allowed to cool to about 20^{o}C and then inoculated with 1kg of Oyster mushroom spores, mixed to form the substrate, and then poured back into the 6 kg plastic bucket. The 6 kg bucket was covered with a dark polythene film and immediately transferred to the dark room to stand for about 6-10 hours.

A total of 6 x 1kg sterile black polythene bags were wet and filled tightly with the prepared substrate. The open ends of the filled bags were tied with masking tape. A sharp sterile stainless steel kitchen knife was used to pierce small holes on each tightly packed bag in about eight (8) places to allow for limited amount of airflow for the spores to germinate into individual mycelium.

The tightly packed bags were transferred to a dark and cool room (<12^{o}C), away from direct sunlight. After about 8 days, the spores germinated into mycelium and spread in the bags, making the mushroom medium to appear white. As a result, the bags were immediately transferred to the second room, which was well ventilated with direct sunlight and its temperature maintained at 23±2.0^{o}C, while the humidity was maintained at 80 ±5.0%.On the 10th day Oyster Mushrooms fruited significantly, and started growing out of the holes initially created on each bag. Growth was observed for about 5-6 days. The mushroom tops spread out wider and bigger in size from the 12th day and were harvested on the 15th day. Harvesting was carried out immediately corresponding to the 15th day of the cultivation process. Harvesting was done by twisting the stem once in one direction. The harvested mushrooms were packed in 1kg sterile transparent polythene bags and stored under refrigeration. A single inoculant gave a total of four (4) harvest times over a period of 34 days of the cultivation process. The first harvest samples and the final and last harvest samples were pooled and analyzed for phytochemical compounds, proximate composition, vitamins and mineral elements concentrations, heavy metals and anti-nutrients (Tables 1-6)..

Sample preparation for analysis of phytochemical compounds, proximate composition, mineral elements, vitamins, heavy metals and anti-nutrients composition.

500g of Oyster Mushroom was washed, cut into small pieces and freeze-dried for 6hr. The dried samples were homogenized using a laboratory mortar and piston. The powdered sample was stored in a sterile polythene bag, from where samples were taken for the determination of proximate composition, mineral elements, vitamins, phytochemical constituents, heavy metals and anti-nutrients.

Quantitative Analysis of Phytochemical Constituents

Phytochemical analysis for saponins, polyphenols, flavonoids, alkaloids, carbolactones, carotenoids, sesquiterpenoids and cardiac glycosides were carried out according to known and standard methods (Igile et al. 2013). Saponin content was determined using the method of Birk et al. (1963) as modified by Achinewhu (1983). Flavonoids, alkaloids and sesquiterpene lactones were determined by Column chromatography fractionation on silica 368 mesh, followed by ethyl acetate extraction and gravimetric measurement, and the alkaline precipitation and gravimetric method, and the double extraction and gravimetric measurement, respectively as described by Harbone (1973).

Analysis of Proximate Composition

The analysis of the proximate composition of the powdered sample of the Oyster Mushroom was carried out using the official methods of analysis of the Association of Official Analytical Chemists (AOAC, 1984), and the FAO (1986).

Analysis of Anti-Nutrients

Tannins were estimated using the Folin-Denis spectrophotometric method (Pearson, 1976). Total oxalate was determined according to the procedure of Day and Underwood (1986). Phytate content was determined using the method described by Reddy and Love (1999). Hydrocyanic acid content was determined using the alkaline titration method of AOAC (1990).

Analysis of Vitamin Composition

The composition of the water-soluble vitamins, including thiamine, riboflavin, niacin, pyridoxine, biotin, Folic acid and cyanocolamin, were determined in powdered Oyster mushroom, using the HPLC method (Jaworska et. al. 2015; Furlani and Godoy, 2007). The composition of the water-insoluble vitamins was further confirmed by the method described by Scalar (2000). Total Vitamin C (ascorbic acid and dehydroascorbic acid) content was determined by the UV-Spectrophotometric method described by Desai and Desai (2019). In this method bromine water oxidized ascorbic acid in the Oyster Mushroom sample to dehydroascorbic acid in the presence of acetic acid. The extract was filtered and allowed to cool. The extract was then coupled with 2,4-dinitrophenyl hydrazine at 37^{o}C for 3 hr, and the solution was treated with 85% H_2SO_4 to produce a red coloured complex. The absorbance of this complex was spectrophotometrically measured at 521nm wavelength. The Vitamin C recovery from Oyster Mushroom was calculated to be 78%. Total Vitamin A concentration was determined by the spectrophotometric method described by Al-Sulimany and Townshend (1973). In this method, iodine was used as the chromogenic agent in the presence of 1,2-dichloroethane, which prevents interference from Vit D_2 and β-Carotene. Vitamin A concentration was further confirmed and compared with the spectrophotometric method described by Pearson (1976). Total Vitamin E concentration in the Oyster Mushroom sample was determined using the Novel HPLC method described by Katsanidis and Addis (1999).

Analysis of Mineral Elements and Heavy Metals

The Atomic Absorption spectrometry (AAS) method was used for the analysis of mineral elements and heavy metals. Ig of the powdered sample of Oyster mushroom was placed in a crucible and ignited in a muffle furnace at 300oC for 6hr. The ashed sample was allowed to cool to room temperature (30±2.0^{o}C). It was then digested with a mixture of 20ml 4M HNO_3 and 60% perchloric acid. The digested sample was diluted with 100 ml of de-ionized water. An aliquot of the diluted sample was analyzed for Fe, Zn, Se, Mn, Co, Li, Sr, Cr, Na, K, Mg, Cu, Ni, by atomic absorption spectrophotometric method described by James (1995) and the AOAC (1990) .

Statistical Analysis
All determinations were done in triplicates and results were expressed as Mean ± SEM, for n=3, and subjected to analysis of variance (ANOVA) to check for statistical significance ($p<0.05$).

RESULTS AND DISCUSSION

A wide variety of Mushrooms grow in the hot, humid and wet regions of the world including Southern Nigeria. Tropical climate has been found to be very favourable for the growth and cultivation of mushrooms including Oyster mushroom (*Pleurotus ostreatus*). The humidity (80.00 ±5.0%), controlled temperatures ranging from (<12.00±2.00 ºC – 20.00±2.00 ºC), and the general weather conditions (long rainy seasons with intermittent sunshine, wind speed & wind direction) in the tropics and sub-tropical regions of the world including Nigeria, are some of the conditions that favour the cultivation of mushrooms. These conditions were optimized in this study for the cultivation of *Pleurotus ostreatus*. Oyster mushrooms have been successfully cultivated in the CRC facility in Calabar, using rubber wood sawdust as growth nutrient, while cultivation conditions were optimized at every stage of the process. The utilization of rubber wood sawdust for this purpose was regarded as an action aimed at degradation of sawdust (environmental biomass), clean-up, bio-remediation and environmental management of the heaps of sawdust littered in the rubber wood processing area. After 15 days of cultivation, the mushrooms were found to be mature and fully fruited.

The results of the harvested mushrooms and phytochemical composition, anti-nutrients and macro and micro-nutrients composition are compiled and presented in Figure 1 and Tables 1-6 respectively. Figure 1 shows the picture of the fully fruited Oyster mushroom harvested on the 15th day after cultivation. The colour was a mixture of white to lilac-gray on the caps while the stalks were white colour (Figure 1). This is consistent with the previous description given by Eger et. al.(1976).

The phytochemical compounds detected in the fruited sample of the Oyster mushroom included polyphenols, saponins, flavonoids, alkaloids, triterpenoids, steroids and carotenoids. Saponins (0.13 ± 0.02 mg/100g), and carotenoid (0.48 ± 0.15 mg/100g) concentrations were significantly higher ($p<0.05$) than all other phytochemicals present in the OM sample (Table 1). It was thought that these compounds migrated into the mushroom from the base nutrient (rubber wood sawdust), and bio-accumulated in the fully fruited tissues of OM. There were no significant ($p = 0.05$) differences in phytochemicals concentrations between the first harvest (on the 15th day), and the final harvest (on the 34th day).

The flavonoids, carotenoid and polyphenols in oyster mushroom may exert antioxidant properties synergistically with the high concentrations of Vitamin C and Vitamin D (Table 3),

and the presence of the antioxidant polysaccharide (pleuran) in the mushroom which has been reported to cure colon cancer (Bobek and Galbavy, 2001).. Pleuran also possesses anti-inflammatory, anti-ulcer, anti-tumour and anti-carcinogenic activities (Patel et.al. 2012). The phenolic, polyphenolic compounds as well as Vitamins C and E in Pleurotus ostreatus, are a potent hepatoprotective, antioxidative and anti-inflammatory agents which will be beneficial to the consumer.

The low fat content, high protein and fibre concentrations found in this study, together with the significant polysaccharide and glycoprotein concentrations previously reported in oyster mushroom (Windholz, 1983; Miyazawa et.al. 2008); and the presence of sugar-lowering molecule (guanide); may significantly contribute to the sugar lowering and anti-diabetic properties of *Pleurotus ostreatus*. . The low fat content and high fibre concentration found in this study further supports the previous findings that diets containing fresh fruiting bodies of Oyster mushrooms or the dried and powdered material significantly lowered plasma lipids and cholesterol in humans and experimental animals (Bobek et. al. 1991; Hossain et. al. 2003).

Figure 1: Harvested Sample of Oyster Mushroom (*Pleurotus ostreatus*) after 15 days of cultivation

The proximate composition of macro-elements present in the sample from this study did not show significant ($p<0.05$) difference between the first and final harvests (Table 2). There was however 2.64% increase in carbohydrate content on the final harvest day 34. There was a corresponding increase (3.41%) in protein concentration on the 34th day. The slight increases observed for carbohydrate and protein concentrations may be attributable to maturity of the

cultivated *Pleuurotus ostreatus* with age. The energy value (308.08±5.44 - 316.01±4.75 kcal/100g) of the harvested mushroom is low, and together with the low fat and high protein concentration may significantly contribute to the anti-obesity properties of *Pleurotus ostreatus* in humans.

The present study showed that the cultivated oyster mushroom on rubber wood saw dust gave very significant concentrations of a wide range of vitamins (Table 3). There were no significant differences in the values of vitamins obtained for the first and final harvests.

Pleurotus ostreatus contain a balanced vitamin concentration, and this is why it is widely reported to exert several nutritional and medicinal properties. It contains significant amounts of antioxidant vitamins C and E (Table 3). aged rats (Jayakumar et.al. 2007). These enzymes are known potent antioxidant enzymes (Keyhani et.al. 2007). It was further reported that, the administration of extracts of *Pleurotus ostreatus* to aged rats resulted in decrease in the concentrations of the oxidative stress enzyme (malondaldehyde), lipids and an electrophilic mutagen due to the antioxidant activities of Vitamin C and E in the mushroom (Buddi et al. 2002).

Table 1: Phytochemical Composition of Oyster Mushroom (*Pleurotus ostreatus*), grown on Rubber Wood Sawdust in Calabar

S/N	Parameter Tested	Result of First harvest (mg/100g)	Result of Final harvest (mg/100g)
1	Polyphenols	0.05 ± 0.01	0.03 ± 0.01
2	Saponins	0.12 ± 0.05	0.13 ± 0.02
3	Flavonoids	0.08 ± 0.02	0.05 ± 0.01
4	Alkaloids	0.04 ± 0.01	0.02 ± 0.01
5	Triterpenoids	0.06 ± 0.02	0.03 ± 0.01
6	Steroids	0.05 ± 0.01	0.04 ± 0.01
7	Carotenoids	0.48 ± 0.15	0.46 ± 0.08

Mean ± SEM, for n=3; Values between groups not significantly ($p \geq 0.05$) different

Table 2: Proximate Composition of Oyster Mushroom (*Pleurotus ostreatus*), grown on Rubber Wood Sawdust in Calabar

S/N	Parameter Tested	Result of First harvest (mg/100g)	Result of Final harvest (mg/100g)
1	Moisture	11.25±1.12	11.96±1.44
2	Total Protein	21.71±1.09	22.45±1.15
3	Fat Content	4.52±0.15	4.77 ± 0.08
4	Total Carbohydrates	[a]44.64±0.61	*45.82±0.45
5	Fibre content	11.42±1.05	11.65±1.52
6	Total Ash	9.55±1.31	9.71±1.09
7	Energy Value (Kcal/100g)	[b]308.08±5.44	*316.01±4.75

Mean ± SEM, for n=3 *Significantly different from a; at $p<0.05$; Values between groups not significantly ($p\geq0.05$) different.

These vitamins have previously been reported to increase the activities of catalase, superoxide dismutase and glutathione peroxidise when extracts of *Pleurotus ostreatus* was administered to Anti-oxidative properties of *Pleurotus ostreatus* commercial mushrooms have recently been reported (Jaworska et. al. 2015). The presence of the B-group vitamins and mineral elements in significant concentrations (Table4), confers a general well being and wellness to the consumer and increases the basal metabolic rate (BMR) of the aged and growing children. The B-group vitamins, Fe, and Zn improve general metabolism, as well as Hb and RBC concentrations in humans. Zn and Se contribute to the development of the brain of infants and children.

Table 3: Vitamin Composition of Oyster Mushroom (*Pleurotus ostreatus*), grown on Rubber Wood Sawdust in Calabar

S/N	Parameter Tested	Result of First harvest (mg/100g)	Result of Final harvest (mg/100g)
1	Vitamin A (IU)	[a]81.22 ± 3.15	*84.75 ± 2.91
2	Vitamin C	27.88 ± 0.65	28.92 ± 1.02
3	Vitamin D	2.92 ± 0.25	2.86 ± 0.40
4	Vitamin E	24.61 ± 0.60	24.55 ± 0.45
6	Vitamin B1	1.84±0.44	1.89 ± 0.50
7	Vitamin B2	2.17±0.53	2.26 ± 0.44
8	Vitamin B6	1.77±0.24	1.81 ± 0.05
9	Niacin	27.01±3.75	26.83 ± 2.40
10	Folic Acid	0.40±0.01	0.44 ± 0.03
11	Pantothenic acid	89.09±5.72	92.31 ± 6.11

Mean ± SEM, for n=3 *Significantly different from a, at $p<0.05$;
Values between groups not significantly ($p\geq0.05$) different

Table 4: Mineral Composition of Oyster Mushrooms (*Pleurotus ostreatus*), grown on Rubber Wood Sawdust in Calabar

S/N	Parameter Tested	Result of First harvest (mg/100g)	Result of Final harvest (mg/100g)
1	Iron (Fe)	[a]56.44±2.7	49.65 ± 3.10
2	Phosphorus (P)	[b]922.05±10.12	*977.23± 15.50
3	Sodium (Na)	13.21±1.22	14.33 ± 0.95
4	Calcium (Ca)	32.17±3.77	34.29 ± 3.05
5	Potassium (K)	[c]1085.09±24.08	*1172.14 ± 29.40
6	Magnesium (Mg)	15.45±1.41	14.66 ± 2.04
7	Copper (Cu)	0.71±0.22	0.67 ± 0.35
8	Zinc (Zn)	3.44±0.95	3.65 ± 0.50
9	Manganese (Mn)	1.55±0.42	1.61 ± 0.08
10	Selenium (Se) µg/100g	5.0±0.02	6.11 ± 0.12
11	Chloride (Cl)	17.44±3.25	19. 61 ± 2.09

Mean ± SEM, for n=3; *Significantly different from a,b,c; at $p<0.05$;
Values between groups not significantly ($p\geq0.05$) different

The level of Vitamin D is far higher than the level in most vitamin D-containing foods and similar to the daily requirement of Vitamin D recommended internationally. This supports the findings that mushrooms may be the only non-animal source of Vitamin D with a concentration that satisfies WHO Vitamin D requirements in a single serve (Cardwell et al. 2018).

Table 5: Heavy Metal Composition of Oyster Mushrooms (*Pleurotus ostreatus*), grown on Rubber Wood Sawdust in Calabar

S/N	Parameter Tested	Result of First harvest (mg/100g)	Result of Final harvest (mg/100g)	EU Specified Limits (mg/kg)
1	Mercury (Hg)	0.003±0.001	0.002±0.001	0.02
2	Cadmium (Cd)	0.003±0.01	0.003±0.001	0.20
3	Lead (Pb)	0.06 ±0.02	0.05 ±0.02	0.30
4	Arsenic (As)	0.002 ± 0.001	0.002 ± 0.001	-----
5	Chromium (Cr)	0.05 ± 0.02	< 0.04 ± 0.02	0.20

Mean ± SEM, for n=3 ND = Not detected
Values between groups not significantly ($p \geq 0.05$) different

The present studies found heavy metals concentrations within regulatory permissible limits (Table 5). The presence of heavy metals in mushroom and particularly *Pleurotus ostreatus* is a major problem to commercial mushroom growers the world over. Probably the concentration of heavy metals in oyster mushroom may depend more on substrate type, soil type upon which the substrate was initially grown, soil geology and hydrology, and the level of contamination of the area by industrial activities and automobile traffic.

Table 6: Anti-nutrients Composition of Oyster Mushrooms (*Pleurotus ostreatus*), grown on Rubber Wood Sawdust in Calabar

S/N	Parameter Tested	Result of First harvest (mg/100g)	Result of Final harvest (mg/100g)
1	Phytate	0.004 ± 0.001	0.003 ± 0.001
2	Oxalate	0.04 ± 0.01	0.03 ± 0.01
3	Cyannate	0.02 ± 0.01	0.02 ± 0.01
4	Tannate	0.23 ± 0.05	0.24 ± 0.03

Mean ± SEM, for n=3 ND = Not detected
Values between groups not significantly ($p \geq 0.05$) different

The CRC is located in a light industrial zone with minimal pollution releases, and very light traffic. The heavy metals profile may have come from the rubber saw dust used as the substrate.
The anti-nutrient concentrations found in the present study (Table 6), are consistent with the levels generally found in edible vegetables. The concentrations of the anti-nutrients were not high enough to interfere with enzyme activity, or with the metabolism and bioavailability of vitamins and mineral elements in humans.

CONCLUSION

The present study has established that *Pleurotus ostreatus* can be grown commercially in Calabar, South-South, Nigeria, under controlled and optimized cultivation conditions, and the nutrient composition did not vary between harvest times. We conclude that rubber wood saw dust proved an excellent base substrate for growing Oyster Mushroom in commercial scale, and the quality of Oyster mushroom harvested met national regulatory standards, and international quality standards (EU specified limits) especially with respect to heavy metals contamination, and CRC should commercialize their product outside the borders of Nigeria.

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Conflict of Interest
The authors have no conflict of interest to declare