Proximate composition, essential heavy metal concentrations and nutrient density of the mycelium and fruiting bodies of organically cultivated *Pleurotus ostreatus*

ABSTRACT

Proximate composition, essential heavy metal concentration of fruiting bodies (POFB) and mycelium (POMY) of *Pleurotus ostreatus* were conducted using standard methods. Moisture content was higher (9.79%) in POFB than in POMY (8.76%) on dry weight basis. Ash was higher in POFB(6.25%) than in POMY(3.25%).POFB presented higher crude protein value (24.66%) than POMY (21.17%).Crude fat values were low in both samples(POFB:0.28%; POMY:0.46) respectively. Fiber was higher in the mycelium (14.72%) than in the fruiting bodies (12.90%). The carbohydrate content of the mycelium was 51.93% while the value of carbohydrate in the fruiting bodies was 46.10%. POFB indicated energy value of 285.60Kcal/100g and the value in POMY was 296.57Kcal/100g. Iron, copper, manganese and chromium presented higher values in POFB than in POMY but zinc indicated higher level in POMY than in POFB. The %DV highlighted in the study showed that the fruiting bodies and mycelium of organically cultivated *P.ostreatus* are rich in protein, fiber, carbohydrate, copper and iron. The nutrient density (ND) results revealed that POFB and POMY samples are nutritionally dense in fiber, copper and iron based on the World Health Food Rating System. The results showed that POFB and POMY obtained by organic cultivation possess high nutritional profile, suggesting that people may use them in food and medicinal formulations.

(Key words: Proximate composition, essential heavy metals, percentage daily value, nutrient density , *Fruiting bodies, mycelium, Pleurotus ostreatus*)

INTRODUCTION

There is no doubt that people strive to survive by seriously engaging in searching for food, substances of biological importance or medicinal substances. Mushrooms such as the Oyster mushroom are edible fungi and they are placed under the class of vegetables in world foods (Maurya *et al* ., 2018) and they fit in very well with sustainable farming systems in both developed and developing nations, possessing huge economic, medicinal as well as nutritional importance. According to Raman *et al*., (2021), mushrooms of the *Pleurotus* family are widely cultivated around the globe for commercial purposes. They are found in everywhere within the tropical rain forests and often grow on fallen dead and decaying tree stumps, as well as wet logs (Raman et al ., 2021). However, Okoroh *et al*., (2019) highlighted that organic cultivation of *P.ostreatus*, particularly by diverse organic supplementation techniques helps to improve its nutritional quality and could be adopted as a method used in enriching mushrooms for feed, food and medicinal formulations. *P. ostreatus* cultivated by organic supplementation method are rich sources of nutrients and could be used in making supplements (Okoroh *et al*., 2017). For many years, humans have utilized mushrooms as source of feeding and for the purpose of healing (Maria *et al.,* 2014). Mushrooms have been taken as small medicinal factories that nature has made .They have been revealed to be rich in immense array of new constituents that humans are yet to tap (Guggenheim, Wright and Zwickey, 2014). . People trade these substances as supplements in diets because they believe that these substances have properties that may improve the human immune system and may also block the formation of cancer tumor (Finimundy *et al.*, 2013) Mshigeni and Chang (2000) highlighted that people who are oriented towards health now enjoy new foods which come from mushrooms and these edible substances got from macro fungi make up the foods that are growing at a very rapid rate around the globe. *Pleurotus ostreatus* belongs the family of mushrooms known as *Pleurotaceae* (Kuo, 2005).*P.ostraetus* is also called tree oyster mushroom (Stamets, 2000) or grey oyster mushroom and this name marks it out from the other species in the genus. Some people call it straw mushroom. The people from Japan call *Pleurotus ostreatus* Hiratake which implies flat mushroom (Hall,2010).The Igbo-speaking people of South-East, Nigeria, call it Ero atakata because it has very tough texture on mastication (Akpaja *et al.,* 2003). *Pleurotus ostreatus* is one of the most common mushrooms that local people hunt from the wild. The macro fungi can also be cultivated by people using saw dust as substrate. The mushroom has bitter sweet aroma of benzoic aldehyde (Beltran-Garcia *et al.*, 1997). Its cap is broad with a fan-like shape. The caps pans from 5cm to 25cm. The macro fungi have colors ranging from white, gray, and tan to dark-brown. At a very tender stage, it has in-rolled margin .Feeling the texture, the mushroom is smooth. It is often lobed. The flesh is white and its thickness varies because of the way its stipe is arranged. The gills may be white or cream in color. The gills normally descend on the stalk. *Pleurotus ostreatus is* edible, medicinal and also very common. A lot of people dwelling in developing nations such as Nigeria still harbor fear to consume wild edible mushrooms because of toxicity and many are still ignorant of organically cultivated mushrooms. The scientific study was aimed at the comparative evaluation of the proximate composition, essential heavy metal concentrations and nutrient density of the mycelium and fruiting bodies of organically cultivated *Pleurotus ostreatus*. The scientific data from this research will help to educate the local populace on the huge economic, nutritional and medicinal potential of organically cultivated *Pleurotus ostreatus* so as to enhance its biodiversity.

MATERIALS AND METHODS

Mushroom Materials

Fruiting bodies and mycelium of *Pleurotus ostreatus* used in this research were purchased from The University of Port Harcourt Mushroom Demonstration Farm (MDF) in May, 2021. They were cleaned, partly dried , packaged with airtight cellophane, labeled and kept for further use.

Sample Preparation for analysis

The samples were dried in the oven at 80^oC for 3 hours and ground to powder using manual grinder. The fine powdered samples were stored in the desiccator and employed for chemical composition analysis.

Analysis of Sample

Moisture, fiber, crude protein, ash and crude fat were analyzed using the Methods of Association of Official Analytical Chemists (AOAC, 2019). Total carbohydrate was estimated using different calculation (Onyeike and Acheru, 2002). Calorific value was obtained by physical scoring via the multiplication of the mean values of total carbohydrate, crude fat and crude protein by the Atwater factors of 4, 9, 4 respectively, taking the sum of the products and expressing the result in kilocalories per 100g sample as described by Onyeike and Ehirim (2002), Essential heavy metals such as iron, zinc, copper, manganese and chromium were determined by atomic absorption spectrophotometry as reported by AOAC (2019). All sample concentrations were obtained in parts per million (PPM) and reported as mg/kg dry weight of sample using a conversion factor of 10 to multiply the concentrating in PPM. (i.e. concentration (mg/kg). = Concentration (PPM) X 10). Percentage daily values (%DV) were determined by comparing the current samples with a 2,000 calorie reference diet, for adults and children aged 4 and above (Nutritional Data, 2011). It was calculated as follows;

Percentage daily value (%DV) = $\frac{\text{Amount A}}{\text{R D A}} \text{x} \frac{100}{1}$,Where A = amount ; RDA = Recommended daily allowance A = amount (i.e. weight of the particular nutrient in a specified quantity of the sample).The nutrient density values were calculated by using the Index of Nutritional Quality (INQ) rating system .It was calculated as follows ;

Nutrient Density = $\frac{\text{Amount of Nutrient/100g} \div \text{RDA for nutrient}}{\text{Calories/100g of food} \div \text{RDC intake.}}$,Where RDA = Recommended Daily Allowance; RDC = Recommended Daily Calorie.

Statistical analysis

Data obtained was statistically analyzed using a one-way analysis of variance (ANOVA) using SPSS/PC+ Package. Differences between means were compared by Fisher's Least Significance Difference (LSD). Significance was accepted at a p – value of less than 0.05 ($P \leq 0.05$)

RESULTS AND DISCUSSION

Proximate composition of the fruiting bodies and mycelium of *Pleurotus ostreatus*

The findings of this study indicated that the moisture content ranged from 8.76% (POMY) to 9.79(POFB) on dry weight basis (Table1). The moisture content of *P.ostreatus* reported in this study was lower than those highlighted by Okoroh *et al*.,(2021) for seeds of *C.lanatus*, Okoroh *et al.,* (2019) for *F. capensis* leaves, Okoroh and Onuoha (2019) (for heat processed seeds of peeled and unpeeled *A.hypogaea* and *A.occidentale*) and Okoroh *et al.,* (2018) for *P.ostreatus* samples cultivated by substrate organic supplementation. Jonathan *et al*., (2006) reported high moisture value in mushrooms in their study. Fresh mushrooms usually contain high moisture content .The environmental condition and the time of harvest could be reasons for the high level of water in the fresh macro fungi. Dry mushrooms usually contain very little amount of water. Foods high in moisture content encourages the growth of microorganisms thereby causing high rate of organic decay (Brock et al.,1986). This implies that too much water in mushrooms will reduce their shelf life. According to Olutiola *et al*., (1991), the amount of water in food shows their water activity. Water activity could be employed to measure how stable and how susceptible these food substances are to microbial contamination (Uriah and Izuagbe, 1990). Moisture content is one of the most important characteristics in consumer sensory perception of food. Change in moisture content will dramatically affect flavor and texture as well as physical and chemical properties, as water gives chemicals a helpful medium to catalyze chemical reactions. Moisture also helps in the digestion. One way to increase the shelf life of the mushrooms is by drying them to reduce its moisture content which in turn reduces microbial activity. The amount of food nutrient in the mushroom will also relatively increase because of drying (Okoroh *et al*., 2018). The fruiting body of *Pleurotus ostreatus* had higher ash content (6.25%) which was significantly different ($p \leq 0.05$) from the value obtained from the mycelium (3.26%). Okoroh *et al*., (2018) indicated higher ash content (7-10%) in samples of *P.ostreatus*, Sumaria *et al*., (2016) showed similar values of ash content for *Pleurotus ostreatus in* their work but Okoroh and Onuoha (2019) indicated lower values of ash for A.hypogae and A.occidentale peeled and unpeeled seeds. Ash content determination in foods is a reflection of the amount of minerals in food. It is important because the amount of minerals can determine physiochemical properties of foods, as well as retard the growth of microorganisms. Therefore, mineral content is a vital component in a food's nutrition, quality and microbial viability. This study reveals that the mycelium had lower ash content than the fruiting bodies. The crude protein content of the cultivated *Pleurotus ostreatus* mushrooms was higher in the fruiting body (24.66%) than in mycelium (21.17%). Okoroh *et al.,* (2017) reported lower values for *P.ostreatus* samples (SAWCS, WWS and AVOS) cultivated by substrate organic supplementation while Mattila *et al.,* (2002) , Okoroh *et al*., (2021), Okoroh and Onuoha (2019) reported comparable values for

P.ostreatus fruiting bodies , *C.lonatus* seeds , and *A.hypogaea* and *A.occidentale* respectively. Proteins are essential macronutrients needed for tissue growth and development as well as major components of enzymes. This implies that daily consumption of *P.ostreatus* fruiting bodies and the use of the mycelium in the preparation of supplements will enhance the health of people. The fruiting bodies and the mycelium of *Pleurotus ostreatus* showed low fat content. The fruiting bodies of *P.ostreatus* had lower fat content (0.28%) than the mycelium (0.46%). Okoroh *et al* ., (2017) highlighted higher values for SAWCS, WWS and AVOS (Species of *P.ostreatus* cultivated by organic supplementation techniques).The crude fat content reported in this study are in consonant with the range reported for most mushrooms (Yang et al., 2001, Mau et al., 2001,). The fruiting bodies and mycelium of *P.ostreatus* are therefore healthy for consumption and should be included in the diets of obese or diabetic patients. Fiber was higher in the mycelium (14.72%) and significantly ($p \leq 0.05$) lower in the fruiting bodies (12.90%) of *P.ostreatus* analyzed. Okoroh *et al*., (2017) reported lower fiber values in SAWCS, WWS and AVOS samples of *P.ostreatus* in their work while Okoroh *et al*., (2021), Okoroh and Onuoha (2019) reported lower value of fiber for *C.lanatus* and *A.hypogaea* and *A.occidntale* processed seeds respectively. Fiber consists of non-nutrient substances such as lignin and cellulose. These materials cannot be digested in the small intestine humans. Nutritionally, fiber is essential because it helps to clean up the intestinal tract and also maintains the movement of the intestine in a peristaltic way (Mukhopadhyay and Guha, 2015). Fiber helps to lower the rate of absorption of glucose in the digestive tract. It also inhibits cholesterol synthesis. The findings in this study indicated that the fruiting bodies and mycelium of organically cultivated *P.ostreatus* are rich in fiber and therefore good to be included in the diets of diabetic patients. Fiber is useful for weight control. Mathenge (1997) reported that fiber is essential in diets because it helps to maintain bulk and the movement of the intestine by peristalsis via surface extension of food in the digestive tract. Excessive consumption of fiber may cause intestinal irritation and may also decrease the availability of other nutrients. On dry weight basis, carbohydrate value of mycelium (51.93%) was higher than the value of the fruiting bodies (46.10%) analyzed. Higher carbohydrate content was reported by Hung and Nhi (2012) and Okoroh et al., (2017) respectively in their study for *P.ostreatus* but Okoroh and Onuoha (2019) reported lower carbohydrate values for processed seeds of *A.hypogaea* and *A.occidentale*. The calorific value of the mycelium and the fruiting bodies of *P.ostreatus* indicated in this study were low. The low calorific value could be attributed to the high fiber, low water and low fat content of the mycelium and fruiting bodies of *P.ostreaus* analyzed in this study (Zahid, Barua and Huq, 2010). The calorific value for the mycelium was higher (296.6kcal/100g) than the value for the fruiting bodies (285.6kcal/100g) of *P.ostreatus*. Sumaira *et al.*, (2016) highlighted lower energy content for *Pleurotus ostreatus* but Okoroh *et al.*, (2017) indicated higher value for SAWCS sample of *P.ostreatus* and comparable value for AVOS sample of *P.ostreatus* in their work. Okoroh and Onuoha (2019) also reported higher energy values for processed *A.hypogaea* and *A.occidentale* which was attributed to the fact that the later are oil seeds.

Table 1: **Proximate Composition (%) Of The Fruiting Body and Mycelium of Organically Cultivated *Pleurotus ostreatus***

Composition	POFB	POMY
Moisture	9.79±0.05[a]	8.76±0.34[b]
Dry matter	90.21±0.05[b]	91.24±0.34[a]
Ash	6.25±0.05[a]	3.26±0.03[b]
Crude protein	24.66±0.22[a]	21.17±0.54[b]
Crude fibre	12.90±0.53[b]	14.72±0.03[a]
Fat	0.28±0.01[b]	0.46±0.02[a]
Total carbohydrate	46.10±0.65[b]	51.93±0.0.36[a]
Calorific value(Kcal/100g)	285.60±1.91[b]	296.57±0.89[a]

Values are means ± standard deviations of triplicate determinations. Values in the same row having different superscripts are significantly different (p≤0.05). Where POFB= *Pleurotus ostreatus* fruiting body, POMY= *Pleurotus ostreatus* mycelium.

Essential heavy metal composition.

Iron was the highest essential heavy metal found in the fruiting bodies and mycelium of organically cultivated *P.ostreatus* analyzed in this study. The fruiting body contained higher Fe level (2.7mg/kg) than mycelium (2.61mg/kg) and the values were significantly different (p≤0.05). Higher values of Fe was reported for *P.ostreatus* samples(SAWCS, AVOS and WWS) cultivated by substrate organic supplementation (Okoroh *et al*., 2017) but Okoroh et al., (2019) and Okoroh et al., (2014) reported lower values of Fe for *F.capensis* and *L.africana* in their study. Iron is needful in the synthesis of blood hemoglobin. This implies that the inclusion of both the mycelium and fruiting body in diets will help build the blood of patients suffering from anemia. Zinc had the second highest composition after Fe and the composition of Zn in the mycelium was slightly higher (1.52mg/kg) than in fruiting bodies (1.50mg/kg), although there was no significant difference (p ≥0.05) between them. Sumaira *et al*., (2016), Okoroh *et al*., (2014) and Jonathan *et al*., (2006) reported lower concentrations of Zn for *P.ostreatus* , *H.crinata* and *P.florida* respectively. Zinc is required for catalysis as well as regulatory functions. Biological systems need zinc for maintaining structure (Cousins et al., 1996). Zinc is also needed to enhance insulin sensitivity. This means that the mycelium and fruiting bodies of *P. ostreatus* could serve as nutritional supplement useful for medicinal purposes. The copper content of the fruiting body was significantly (p≤0.05) higher (0.48mg/kg) than the copper content in the mycelium (0.36mg/kg). Oxidation-reduction reaction in metabolic processes which include mitochondrial tissue respiration, cross-linkage formation by collagen and melanin formation needs copper as an essential trace element. Copper-zinc superoxide dismutase contains copper as a major component. Superoxide dismutase is an important enzyme in anti-oxidation to preserve biological system from free radical effect (WHO, 1998). The fruiting bodies showed higher manganese content (0.16mg/kg) than the mycelium (0.12mg/kg) and the values were significantly different (p≤0.05). Manganese is an important enzyme cofactor (Crook, 2012).

Manganese is essential for human health because it is necessary for metabolism, cell development as well as antioxidant systems (Emsley, 2001). However, excess manganese in biological system may result to a neurodegenerative disorder called manganesm. An adult male requires manganese reference intake of about 2.2mg in a day. This implies that the consumption of *P. ostreatus* mycelium and fruiting body could meet daily requirement of the trace mineral when consumed in large quantity. Chromium in fruiting bodies (0.14mg/kg) was higher than in POMY (0.06mg/kg) and these values were significantly different ($p \leq 0.05$). Okoroh *et al*.,(2017) and Okoroh et al.,(2019) reported higher value of chromium for *P.ostreatus* samples (AVOS, SAWCS and WWS) and *F.capensis* respectively in their study but Okoroh *et al*., (2019) reported a lower value of chromium for *L.africana*, *H.crinata* and *V.amygdalina* respectively.

Table 2: Essential Heavy Metal Composition (mg/kg) of Fruiting bodies and mycelium of organically Cultivated *Pleurotus ostreatus.*

Analyte	POFB	POMY
Fe	2.79±0.01[a]	2.61±0.01[b]
Zn	1.50±0.03[a]	1.52±0.01[a]
Cu	0.48±0.01[a]	0.36±0.01[b]
Mn	0.16±0.01[a]	0.12±0.02[b]
Cr	0.14±0.01[a]	0.06±0.03[b]

Values are means ± standard deviations of triplicate determinations. Values in the same row having different superscripts are significantly different ($p \leq 0.05$). Where POFB= *Pleurotus ostreatus* fruiting body, POMY= *Pleurotus ostreatus* mycelium.

The results of the proximate nutrient potential (percentage daily value) and essential heavy metal nutrient potential (percentage daily value) of the fruiting bodies and mycelium are highlighted in tables 3 and 4. The %DV for crude fiber was highest followed by that of crude protein and the lowest was that of crude lipid potential, considering the proximate nutrient potential of the samples analyzed. Okoroh *et al* (2018) reported lower crude fiber potential, crude protein potential, carbohydrate potential, energy potential but higher fat potential than the values reported in this study. Copper had the highest %DV followed by iron but the value of chromium was very low in both samples of P.ostreatus. Percentage daily values of foods give an in-depth nutritional profile of diets and they are very vital in drafting nutritional information. The values highlighted in this study confirms that the fruiting bodies and mycelium of organically cultivated *P.ostreatus* are rich in protein, fiber, carbohydrate, copper and iron. Nutrient potential compares the amount of nutrient in the sample to the recommended daily allowance. The iron potential reported in this study is in consonant with the value reported by Okoroh *et al*., (2018) for samples of *P.ostreatus* cultivated by substrate organic supplementation, the values of manganese and zinc were lower, the values for copper were higher than those reported by Okoroh et al., (2018) for samples of *P.ostreatus* studied but the values for chromium were comparably low.

Table3: Proximate nutrient potential of the fruiting bodies and mycelium of organically cultivated *P.ostreatus*.

Percentage daily value (%DV)/100g		
Composition	POFB	POMY
Crude protein	44.08 ± 0.22[a]	37.80± 0.54[b]
Crude lipid	0.64 ± 0.08[b]	1.05 ± 0.02[a]
Crude fiber	51.6 ± 0.53[b]	58.90 ±0.03[a]
Total carbohydrate	30.66 ± 0.65[b]	34.62 ± 0.36[a]
Calorific value	14.28 ± 1.91[b]	14.83 ± 0.89[a]

Values are means ± standard deviations of triplicate determinations. Values in the same row having different superscripts are significantly different (p≤0.05). Where POFB= *Pleurotus ostreatus* fruiting body, POMY= *Pleurotus ostreatus* mycelium.

Table 4: Essential heavy metal nutrient potential of the fruiting bodies and mycelium of organically cultivated P.ostreatus.

Percentage daily value (%DV)/100g		
Analyte	POFB	POMY
Iron	34.88 ± 0.01[a]	32.63± 0.01[b]
Zinc	13.64± 0.03[a]	13.82 ± 0.01[a]
Copper	53.33 ± 0.01[a]	40.00 ±0.01[b]
Manganese	7.27 ± 0.01[a]	5.45 ± 0.02[b]
Chromium	0.4 ± 0.01[a]	0.2 ± 0.01[a]

Values are means ± standard deviations of triplicate determinations. Values in the same row having different superscripts are significantly different (p≤0.05). Where POFB= *Pleurotus ostreatus* fruiting body, POMY= *Pleurotus ostreatus* mycelium.

. The results of the nutrient density of the selected essential heavy metals and fiber are shown in Table 5. The nutrient density of the heavy metals and fiber reported in this study was lower than the values reported by Okoroh and Onuoha (2019) for processed seeds of A.hypogaea and A.occidentale. Nutrient density compares the nutrient potential of food to the energy potential. It is an in-depth nutritional potential that expresses the Nutritional Quality Index of foods. The results from this study highlighted that both samples are nutritionally dense in fiber and copper, iron based on the World Health Food Rating System.

Table 5: The nutrient density of selected essential heavy metals and fiber of fruiting bodies and mycelium of organically cultivated *P.ostreatus*

	POFB	POMY

Composition	%DV	ND	WHFR	%DV	ND	WHFR
Iron	34.88	2.44	good	32.63	2.2	good
Zinc	13.64	0.94	poor	13.82	0.93	poor
Copper	53.33	3.73	V. good	40.00	2.70	good
Manganese	7.27	0.51	poor	5.45	0.37	good
Chromium	0.40	0.03	poor	0.2	0.01	poor
Fiber	51.6	3.61	V.good	58.9	3.97	V.good

Nutrient density values were obtained based on the index of nutritional quality rating system; POFB=*Pleurotus ostreatus* Fruiting Body, POMY=*Pleurotus ostreatus* mycelium, %DV=percentage daily value, ND=nutrient density, WHFR=World Health Food Rating: Excellent (ND≥ 7.6), Very good(ND≥ 3.4), Good (ND≥ 1.5), Poor (≤ 1.5).

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