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PROXIMATE, MACROELEMENT AND TRACE MINERAL COMPOSITION OF THE FRUIT BODIES OF *PLEUROTUS OSTREATUS* (*PLEUROTECEA*) CULTIVATED BY THREE SUBSTRATE ORGANIC SUPPLEMENTATION TECHNIQUES

Okoroh, P.N[1]

[1]Department of Biochemistry, Nutrition and Toxicology Unit, Faculty of Science, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.

Uwakwe, A.A[2]

[2]Department of Biochemistry, Nutrition and Toxicology Unit, Faculty of Science, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.

Monago-Ighorodje, C.C[3]

[3]Department of Biochemistry, Nutrition and Toxicology Unit, Faculty of Science, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.

ABSTRACT

Proximate composition, macroelement and trace mineral concentrations of the fruit bodies of Pleurotus ostreatus cultivated by substrate organic supplementation techniques was determined using standard methods. Avocado seed supplementation (AVOS), whole wheat supplementation (WWS) and soyabean plus Avocados plus wheat plus corn supplementation (SAWCS) were employed. Moisture content was highest in AVOS (13.63±0.06%) and lowest (9.51±0.02%) in SAWCS. Ash was highest 10.10±0.011%) in AVOS and lowest (7.57±0.01%) in SAWCS. Crude protein was highest (17.87±0.02%) in SAWCS followed by WWS (17.03±0.01) and lowest (15.81±0.01%) in AVOS. Total carbohydrate was highest (56.47±0.01%) followed by WWS (56.33±0.02%) and lowest (54.06±0.02%) in AVOS. Crude fibre was highest (7.26±0.02%) in SAWCS, followed by (6.50±0.10%) in WWS and lowest (5.48± 0.01%) in AVOS. Crude fat was highest (1.34±0.01%) in SAWCS and lowest (0.96±0.01) in AVOS. Caloric value was highest. (309.4±0.19%) in SAWCS and lowest (288.1±010%) in AVOS. Macroelements predominant in the samples were K and Mg with values of 3654.3 and 87.8 mg/kg in AVOS, 3661.6 and 88.5 mg/kg in WWS, 3685.9 and 284.7 mg/kg respectively in SAWCS. Trace minerals high in the samples were Fe, Zn, Cu and Mn with values of 70.47, 44.59 and 6.78 and 6.85 mg/kg respectively in SAWCS; 58.87, 37.77, 6.05 and 4.57 mg/kg respectively in AVOS and 53.35, 38.68, 6.03 and 4.95 mg/kg respectively. These findings highlighted the macrofungi samples cultivated by organic substrate supplementation as rich sources of fibre, protein, ash, K, Mg, Fe, Zn, Cu and Mn, hence, may offer scientific basis for the use of the macrofungi as nutritional supplements in food and feed formulations for humans and livestock.

KEYWORDS:*Proximatecomposition, macroelement, trace mineral, fruit bodies, substrate organic supplementation technique*

INTRODUCTION

Comprehensively, "Mushroom is a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand" (Chang and Miles, 1992). There are about 100,000 macrofungi out of which about 150 species are consumable. They are widely used in human diets (Chang 1980). Mushrooms are now known in the whole world and people cultivate it both of subsistence use and commercial use. The high rise in the cost of the major sources of animal protein such as fish, chicken and beef has resulted to the increase in the demand of edible mushroom Aletor (1995); Okwulehie and Odunze (2004).

Mushrooms possess great taste, nutritional qualities and medicinal values as well as numerous application in industries (Lim et al., 2004). People all over the globe consume *Pleurotus* species because they are nutritionally and medicinally good (Pandiarajan et al., 2011). Today, mushrooms are used as tonics with the belief that they have the capacity to enhance the quality of human health (Chang and Buswell, 2008). Mushrooms have medicinal functions which has been attributed to the presence of bioactive substances such as terpenoids, alkaloids, flavonoids, Polyphenols etc (Erjavec et al., 2012). They are popular valuable foods because they contain low levels of calories, sodium, carbohydrates and fats and provide important nutrients such as Se, K, fiber, vitamin B_2, niacin and vitamin D. (Maria et al., 2014). The content, type and concentration of mushroom mycochemicals are influenced by differences in substrate supplementation, cultivation methods, strain, developmental stage, storage conditions, processing, solvent extract etc (Mattila et al., 2002; Barros et al., 2007). According to Feeney et al., (2014), recent research has focused on development of methods to enhance the levels of important bioactive components of cultivated mushrooms so as to improve their nutritional as well as medicinal values. There is no enough scientific information on the proximate, macroelement and trace mineral composition of *Pleurotus ostreatus* cultivated by diverse substrate organic supplementation techniques. This study evaluated the proximate, macroelement and trace mineral composition of *Pleurotus ostreatus* cultivated by three substrate organic supplementation techniques.

MATERIALS AND METHODS

Cultivation of *Pleurotus ostreatus*

The fungal culture used in this study was strain from ready mother spawn (grown on sorghum seeds) from a potato Dextrose Agar (PDA) tissue culture purchased from the Mushroom Research Unit, Demonstration farm, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

Whole grains of wheat, seeds of Avocado pear, seeds of soyabean, maize seeds attached to the cob were sourced from local farms in Rivers State, South-South Nigeria. The materials were ground with electrical grinder and used as organic supplements for the basal substrate (saw dust) and these were mixed with different ratios to get three treatments in many replications.

Calcium trioxocarbonate (IV) and calcium tetraoxosulphate (VI) were added to the treatments followed by the addition of water until a humidity of 68% was obtained. Mixtures were packed into heat resistant polypropylene bags at 1kg per bag. A poly vinyl chloride pipe of 2.0cm thick and 2.5cm long was inserted at the neck of each bag to serve as bottle neck and extra propylene was pulled through the PVC pipe and held in place with rubber band and piece of cotton wool was plugged at the neck of the bags. Substrate bags labeled AVOS, WWS and SAWCS were loaded in a drum for pasteurization by steam generated from boiling water at 100^{o}C for 5hrs. The drum was allowed to cool and the bags transferred to the inoculation room. Ethanol (70% v/v) was used to disinfect the hands, inoculation room, equipment and spawn bottles to prevent cross-contamination and the bags were inoculated by insertion (2 to 2.5cm deep) into the supplemented substrates. Inoculated bags were transferred into the incubation room (using disinfected plastic baskets) for spawn running under complete darkness at controlled temperature of 25^{o}C to 20^{o}C.

Humidity of substrate bags was accomplished by spraying water two times, daily and incubation lasted for 40 days, the bags were colonized as mycelia thickening was observed and the bags were then opened for fruiting after transfer to the fruiting room. Fruiting occurred at optimal temperature of 24^{o}C in the presence of large circulation of air and enough light. Fruiting bodies matured within 48 hours and we harvested and kept in the refrigerator using loosely closed paper bags. They were later dried at 80^{o}C for 24 hours stored in tightly sealed containers for further analysis.

Sample Preparation

For analysis, fruit bodies of *Pleurotus ostreatus* samples were dried in the oven at 80^{o}C 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, fibre, crude protein, ash and crude fat were analyzed using the Methods of Association of Official Analytical Chemists (AOAC, 2006). Moisture content was obtained by heating three 3.0g portions of the mushrooms samples, that has been accurately weighed using a chemical balance, in crucible placed in the oven at 110^{o}C for 1 hour with lid, until a constant weight was obtained. Ash was obtained by incinerating 5g of the sample in a muffle furnace at 500^{o}C for 7 hr.

Kjedahl method based on AOAC (2006) was employed in the determination of nitrogen and crude protein content and percentage nitrogen was converted to crude protein by multiplying percentage nitrogen using the protein conversion factor of 6.25 (i.e crude protein =% Nitrogen x 6.25).

Crude lipid was determined by exhaustively extracting 3 triplicates of 5g of ground sample in the soxhlet extractor using 300ml of anhydrous diethyl ether (B.p range 40-60^{o}C) for 6hr. Crude fibre was determined by hydrolyzing 3.0g of defatted sample in a 1000ml conical flask with 20ml of 1.25% concentrated H_2SO_4 and boiled under control for about 30min using a hot plate. The mixture was filtered by suction and the residue was washed free of acid using hot distilled water followed by boiling of residue in a 1000ml volumetric flask using 200ml of 1.20% NaOH solution for 30min. The residue was once again rinsed with distilled water, finally collected and transferred into a crucible, dried in an oven, to constant weight. The sample was then ashed, in a muffle furnace for 30min.

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 et al., (1995), Macroelements K, Na, Mg and Ca as well as trace minerals Cr, Zn, Mn, Fe, Cu were determined by atomic absorption spectro-photometry as reported by AOAC (2006). 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).

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 ($P<0.05$)

3. RESULTS AND DISCUSSION

The result of the proximate compositions and calorific values of the mushroom are shown in table 3.1. Moisture content was highest in AVOS (13.63±0.06%) and lowest in SAWCS (9.51±0.02%) but values for SAWCS and WWS were significantly different ($P>0.05$). Dry matter ranged from AVOS (86.37±0.06%) to SAWCS (90.49±0.02%). Ash was found to be highest in AVOS (10.10±0.11%) followed by WWS (9.35±0.01%) and lowest (7.57±0.01) in SAWCS. The crude protein in the three samples were significantly different ($P>0.05$) and the value was highest in SAWCS (17.87±0.02% and lowest in AVOS (15.81±0.01%). Crude fat was in the order: SAWCS (1.34±0.01), WWS (1.19±0.01) and AVOS (0.96±0.01%) respectively. Crude fibre was highest in SAWCS (7.26±0.02) and lowest in AVOS (5.48±0.01) and the values were significantly different ($P>0.05$). SAWCS had the highest total carbohydrate (56.47±0.01%) followed by WWS (56.33±0.01%) and lowest in AVOS (54.06±0.02%). Calorific value was in the order: SAWCS (309.4±0.19%), (304.2±0.06%) in WWS and AVOS (288.1±0.10%) and the values were significantly different ($P<0.05$).

Time of harvest and environmental conditions may influence moisture content. The moisture content of mushrooms that are dried ranges from 10-13/1 (Chang and Miles 1989)) and the values obtained in this study is in consonant with the stated value. Moisture enhances the growth of microbes and also increases decomposition rate (Brock et al., 1986); meaning that high moisture value of mushrooms signifies low shelf life and easy decomposition. According to Olutiola et al., (1991), the level of moisture in foods reflect their water activity and can be used (Uraih and Izuagbe, 1990) to measure stability and susceptibility to contamination by microbes. The drying of the mushroom samples reduced moisture level and could serve as measure of preservation of the mushrooms enhancing their shelf life and increasing the relative concentrations of nutrients in them (Table 1).

Sumaira et al., (2016) reported lower values of ash while Hung and Nhi (2012) reported similar ranges to the findings in this study. Ash content is a reflection of the value of minerals in the samples. The values of the crude protein in this study were higher than protein contents of *G.lucidum* (Sumaira et al., 2016) but lower than those of *P.citrinopilaetus* (Fredrick et al., 2013), *L.edodes, H.erinaceus* and *P.ostreatus* (Sumaira et al., 2016). The crude protein values in this study were comparable to the values highlighted by Hung and Nhi (2012) in their work for the mushrooms they analyzed on dry weight basis but the value of crude protein for *Pleurotus ostreatus* (24.6%) revealed by Mattila et al., (2002) was higher. Crude protein values may vary due to developmental stage, availability of nitrogen element, habitat, type of mushroom and nature of supplementation (Calak, Falz and Seslyi, 2009). Since the crude protein values of the samples of *pleurotus ostreatus* analyzed in this study were appreciably high, it implies that they can be consumed in diets as good sources of proteins especially as they contain all the essential amino acids needed by humans.

The low crude fat recorded in this study was similar to the value reported by Fredrick et al., (2013) for *Pleurotus citrinopilaetus* (1.32%) Sumaira et al., (2016) also reported comparable values in their work for *G. lucidum*, *pleurotus ostreatus* and *H.erinaceus*. The

values were also in consonant with the range indicated for most mushrooms (Yang et al., 2001; Mau et al., 2001) and Huang et al., (1985) had already reported a total lipid content of commonly cultivated mushrooms within the range 0.6% and 3.1% of the dry weight and the values observed in this study are within their range. According to Huang et al., (1985), about 72% of the total fatty acids in the crude lipid are unsaturated and they have been revealed to be important to human health and very significant in diets being essential and less dangerous than saturated fatty acids.

Fredrick et al., (2013), Mau et al., (2001) and Yang et al., (2001) presented higher values of fibre while Sumaira et al., (2016) reported crude fibre value in *Pleurotus ostreatus* lower than the values in SAWCS and WWS but higher than the value for AVOS samples according to our findings in this study (Table 3.1).

Lignocellulosic substances and cell wall polymers make up fibre and human cannot digest them. Fibre is good nutritionally because it helps to clean and maintain intestinal motility (Mukhopadhyay and Guha, 2015). Fibre also lowers the rate of absorption of glucose in the digestive tract and binds to cholesterol their by removing it. Since the findings in this study indicated that the mushroom samples are rich in fibre, they are therefore good in the diet of diabetic patients. The carbohydrate values (Table 3.1) recorded in this study were lower than those of *G. lucidum* (82.47%), *H.erinaceus* (76.50%), *L. edodes* (70.62%), *Pleurotus ostreatus* (69.86%) and *V. volvacea* (65.34%) according to the works of Sumaira et al., (2016). Mattila et al., (2002) also reported carbohydrate values for *Pleurotus ostreatus* (62.5%) and *L.edodes* (69.0%) which is higher than our findings in this study but Hung and Nhi (2012) reported lower values of 52.5% for *Pleurotus ostreatus* than the values for WWS and SAWCS respectively in our study. Carbohydrates are sources of energy.

All the samples in this study had calorific values comparable to the values reported by Sumaira et al., (2016) for mushrooms in their work. Mushrooms are generally known to be foods with low energy value as a result of their high fibre content and low fat value (Zahid, Barua and Huq, 2010).

Table 1: Proximate Composition (%) and Calorific Values of the Fruiting Bodies of *Pleurotus ostreatus* Cultivated by Different Organic Supplementation Methods.

Constituent	AVOS	WWS	SAWCS
Moisture	13.63±0.06^a	9.58±0.02^b	9.51±0.02^b
Dry matter	86.37±0.06^b	90.42±0.02^a	90.49±0.02^a
Ash	10.10±0.11^a	9.35±0.01^b	7.57±0.01^c
Crude protein	15.81±0.01^c	17.03±0.01^b	17.87±0.02^a
Crude fat	0.96±0.01^c	1.19±0.01^b	1.34±0.01^a
Crude fibre	5.48±0.01^c	6.50±0.10^b	7.26±0.02^a
Total carbohydrate	54.06±0.02^c	56.33±0.02^b	56.47±0.01^a
Caloric value (Kcal/100g sample)	288.1±0.10^c	304.2±0.06^b	309.4±0.19^a

Values are means ± standard deviations of triplicate determinations. Values in the same row having the same superscript letters are not significantly different ($P<0.05$). Where AVOS = Avocado seed supplementation, WWS = whole wheat supplementation, SAWCS = soyabean plus Avocado seed plus while wheat + corn/cob supplementation.

The result of the macroelement levels of the mushrooms is shown in table 2. The result highlighted the concentrations of the macroelements from the highest value to the lowest value in the three samples, in the order: potassium (3685.9mg/kg in SAWCS; 3661.6mg/kg in WWS, 3654.3mg/kg in AVOS); Magnesium (288.5mg/kg in WWS, 287.8mg/kg in AVOS, 284.7mg/kg in SAWCS); sodium (171.7mg./kg in WWS 149.5mg/kg in SAWCS, 117.6mg/kg in AVOS); and calcium (87.27mg/kg in SAWCS, 60.72mg/kg in WWS and 29.92mg/kg in AVOS).

Mallikarjuna et al., (2013) in their study reported high K values on dry weigh basis for *L. Cladopus and P. djamor*, growing in the wild, Sumaria et al., (2016) also reported high range of K in the mushroom they analyzed as well as Jonathan et al., (2006) but all their K. concentrations were lower than the values indicated in our work for all the *Pleurous ostreatus* samples (AVOS, WWS, SAWCS) according to this study. Potassium is an important mineral as an intracellular ion to maintain electrolyte balance (Pohl et al, 2013). Electrical potential difference created across cell membrane by K and Na is necessary for neurotransmission, contraction of the muscle as well as normal function of the heart and its associated blood vessels (Mikko et al., 2006). Lower level of K in the body results to a condition called hypokalemia. Lower levels of K causes hypertension (Aburto et al., 2013). This means that the mushroom samples when put in diets may be used to prevent hypertension linked to low K diets. An RDA of 4700mg of K has been

recommended for adults by the guidelines of the institute of medicine (DRI, 2004). The daily consumption of all the three samples of *Pleurotus ostreatus* could meet the requirement in adults. Potassium aids iron, phosphrous, calcium and magnesium to complete the absorption of vitamins, carbohydrates, fats and proteins (Islam et al., 2004).

All the mushrooms samples analyzed revealed high Mg concentration than the amount needed daily by humans. Sumaira et al., (2016) and Jonathan et al., (2006) reported lower Mg values for the mushrooms, they analyzed than our findings in this study. Magnesium is a very important mineral in protein and nucleic acid production. In organs and systems, Mg is essential for metabolism and specific neuromuscular as well as cardiovascular activities (Ryan, 1991). It aids in permeability characteristics (Noronha and Matuschak, 2002) and electrical properties of biological membranes.

The value of Ca revealed in this study were comparable to the values reported by Jonathan et al., (2006) for certain mushrooms collected in the wild. Higher values of Ca on dry weight basis were reported by Sumaira et al., (2016). The absorption and accumulation of minerals from organically supplemented substrates which served as their habitat could be responsible for the mineral level in them since mushrooms are bioaccumulators. Cell physiology as well as the biochemistry of the cell needs Ca. calcium is involved in signal transduction, contraction of muscle, fertilization, transmission of impulses across the neurons and also plays the role of a second messenger (Brini et al., 2013). A lot of enzymes use Ca as a co-factor, teeth and bone formation requires the mineral and it is also essential in the maintenance of potential difference across excitable cell membrane (Brini et al., 2013). The values of Na in this study were lower than those reported by Jonathan et al., (2006) for wild mushrooms they analyzed. Sodium is essential in acid base balance regulation but when it is in high concentration in the system, it becomes harmful to health. The low concentration of Na in our study for the three mushroom samples indicates that they may be of health value to hypertensive and diabetic patients as well as sufferers of obesity who do not need high sodium levels in their diets.

Table 2: Macro-Element Composition (mg/kg)* of the Fruiting bodies of *Pleurotus ostreatus* Cultivated by Different Organic Supplementation Methods.

Constituent	AVOS	WWS	SAWCS
Potassium	3654.3±0.07^{c}	3661.6±0.07^{b}	3685.9±0.07^{a}
Calcium	29.92±0.02^{c}	60.72±0.01^{b}	87.27±0.01^{a}
Magnesium	287.8±0.13^{a}	288.5±0.01^{a}	284.7±95.47^{a}
Sodium	117.6±0.01^{c}	171.7±9.91^{a}	149.5±0.01^{b}

Values are means ± standard deviations of triplicate determinations. Values in the same row having the same superscript letters are not significantly different ($P<0.05$). Where AVOS = Avocado seed supplementation, WWS = whole wheat supplementation, SAWCS = Soyabean plus Avocado seed plus while wheat + corn/cob supplementation.

The result of the trace mineral concentrations of the mushrooms is shown in table 3. The result indicated that the trace mineral composition of the mushroom cultivated by substrate organic supplementation were as follows: Fe (70.47mg/kg in SAWCS, 58.87mg/kg in AVOS, 53.35mg/kg in WWS); Zn (44.59mg/kg in SAWCS, 38.68mg/kg in WWS, 37.77 mg/kg in AVOS); Cu (6.78mg/kg in SAWCS, 6.05mg/kg in AVOS, 6.03mg/kg in WWS), Mn (6.85mg/kg in SAWCS, 4.95mg/kg in WWS, 4.57mg/kg in AVOS), and Cr (0.001mg/kg in SAWCS, WWS and AVOS respectively); the values of Cu in AVOS and WWS were not significantly ($P<0.05$) different but the concentrations of all other minerals such as Zn, Mn and Fe in the three samples were significantly different ($P>0.05$) except for Cr which was also not significantly different ($P<0.05$) in the samples.

The Fe value of all the samples on dry weight basis were higher than that of all the mushrooms collected from the wild and analyzed by Jonathan et al., (2006), but lower than the value reported by Sumaira et al., (2016) for *V. volvacea.* Iron is essential in heamoglobin formation meaning that the inclusion of these samples of macrofungi in our diets could help build the blood of patients suffering from anaemia.

The concentration of Zn in the samples analyzed were lower than the value reported by Sumaira et al., (2016) for *V. volvacea* and higher than the values reported by Jonathan et al., (2006) for *A. polytricha, P. florida, V. esculanta* and *T. globulus* respectively. Catalytic, regulatory and structural activities in body cells need Zn, (Cousins et al., 1996). The values of Zn recorded in the 3 samples satisfies the daily human Zn intake of 14-30mg/kg/day (Johnson et al., 1993). The mushroom samples could therefore serve as nutritional

supplements for diabetic patients against hyperzincurea and hypozincemia.

Copper is a very essential trace mineral in redox reaction during metabolic processes like mitochondrial cellular respiration, melanin formation and collagen cross-linkaging. Zn is an integral part of superoxide dismutase, an antioxidant enzyme (WHO, 1998). Sumaira et al., (2016) reported a copper level in *V. volvacea* higher than the values of Cu revealed in our study but the levels of Cu reported by Jonathan et al., (2006) were lower.

The value of Mn reported by Sumaira et al., (2016) in *L.edodes* was higher on dry weight basis but Jonathan et al., (2006) revealed Mn concentrations in V. esculanta, *P. atroumbonata* and *P. florida* comparable to the levels revealed in this study. Mn is an enzyme cofactor (Crook, 2012), it is essential for human health, being necessary for metabolism, development and antioxidant system (Emsly, 2001): but excess of the trace mineral may cause a neurodegenerative disorder called Manganism. Mn reference intake for an adult male is 2.2mg/day from diet. The consumption of the mushroom samples can meet the daily requirement of Mn.

Chromium had the lowest concentration amongst all the minerals analyzed in this study and recent reports on the value of Cr in mushrooms from China also confirmed our finding that Cr is few and insufficient in mushrooms. (Xu et al., 2012; Zhang, Cao and Xu, 2012).

Table 3: Trace-mineral Composition (mg/kg) of the Fruiting bodies of *Pleurotus ostreatus* Cultivated by Different Organic Supplementation Methods.

Constituent	AVOS	WWS	SAWCS
Chromium	0.001±0.00	0.001±0.00	0.001±0.00
Iron	58.87±0.00^{b}	53.35±0.01^{c}	70.47±0.01^{a}
Manganese	4.57±0.01^{c}	4.95±0.01^{b}	6.85±0.01^{a}
Zinc	37.77±0.02^{c}	38.68±0.01^{b}	44.59±0.00^{a}
Copper	6.05±0.01^{a}	6.30±0.01^{b}	6.73±0.01^{a}

Values are means ± standard deviations of triplicate determinations. Values in the same row having the same superscript letters are not significantly different ($P<0.05$). Where AVOS = Avocado seed supplementation, WWS = whole wheat supplementation, SAWCS = soyabean plus Avocado seed plus while wheat + corn/cob supplementation.

CONCLUSION

The results in this study highlighted that the three samples of *Pleurotus ostreatus* obtained by substrate organic supplementation cultivation methods were rich in fibre, protein, ash and macroelements such as K and Mg. They also contained high levels of trace minerals such as Fe, Zn, Cu and Mn. The scientific data suggests that the mushroom samples could be included as nutritional supplements in food and food formulations for humans and livestock.

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