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Proximate Nutrient Potential, Phytochemical Screening and Vitamin B-Composition of *Pleurotus ostreatus* Cultivated by Substrate Organic Supplementation Techniques

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OP, AB, NC and EA designed the study. Authors NC, ON, OC, EI and KW performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OP, ON, OC and KW managed the analyses of the study. Authors OP, AB, EI and KW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The proximate nutrient potential, phytochemical screening and vitamin B-composition of *Pleurotus ostreatus* (P.O) cultivated by three substrate organic supplementation techniques (avocado seed supplementation (AVOS), whole wheat supplementation (WWS) and soya bean + avocado + wheat + corn supplementation (SAWCS) were determined using standard methods. Proximate nutrient potential revealed that (SAWCS) had the highest crude protein potential (35.75 ± 0.03) and AVOS the least (31.62±0.02). SAWCS had the highest crude fibre potential (29.0±0.06) followed by WWS (26.91± 0.04) and AVOS the least (21.91±0.02). Qualitative phytochemical screening of the ethanol extract of *P. ostreatus* samples highlighted the presence of alkaloids, triterpinoids and steroids. B_1-vitamins observed and their respective percentage daily values were 0.90 mg/100 g and 7.5% for AVOS, 1.23 mg/100 g and 10.3% for SAWCS; and 2.07 mg/100 g and 17.2% for WWS. Also, B_2-vitamins observed and their respective percentage daily values were 1.60/100 g and 12.3% for AVOS, 0.20 mg/100 g and 1.5% for SAWCS and 1.11 mg/100 g and 8.5% for WWS. These results showed that *P. ostreatus* cultivated by substrate organic supplementation techniques was rich in crude protein, crude fibre, vitamin B_1 and bioactive substances and suggest the employment of the technique in the improvement of mushrooms (during cultivation) for food, feed and medicinal formulations.

Keywords: Proximate nutrient potential; phytochemical; vitamin B-complex; Pleurotus ostreatus.

1. INTRODUCTION

Food materials of plant origin have been treasured for their ability to facilitate organismal growth and total wellbeing [1-13]. Research studies have revealed the importance of phytochemical and nutritional contents in food materials of plant origin [14-17]; their ameliorative effects against health degenerative indices following consumption or administration [11-12,18-20], and general wellbeing of organisms [21-29]. According to Duru et al. [30], there are other existing kingdoms apart from plant and animal kingdoms that can provide food materials for organismal survival.

Mushrooms are amongst these kingdoms and researchers have long keyed into their usefulness as food materials [31-34], as well as their health importance [35-36]. People also have come to treasure mushrooms as food materials that promote health indices. This could be due to their healing potentials in traditional medicine [37]. According to Chang and Miles [38], a mushroom is a macro fungus, with a defined fruiting body, which could be epigeous or hypogenous, with size big enough for the eyes to see and for the hand to pick. Mshigeni and Chang [39], reported that people in developing and developed nations should be encouraged to cultivate mushrooms in large scale and use them to address health challenges. Mushrooms are regarded as small medicinal factory which nature has provided for humans. They are rich in immense array of new compounds which are untapped [40]. There are about 14,000-15,000 kinds of mushroom around the world [41].

Pleurotus ostreatus, a *pleurotecea* mushroom is among the immense array of mushrooms with untapped new compounds [42]. Duru et al. [30]; and Stamets [43] noted that *P. ostreatus* is called Oyster mushroom. It can be found in the wild, and can as well be cultivated using saw dust as substrate [30]. *P. ostreatus* is edible and has tough texture on mastication. It is medicinal. It has been noted that *P. ostreatus* is among the most common mushrooms. It is rich in dietary fibre, proteins, macro and trace elements. Its phytochemical content has made it a special diet for the prevention and treatment of hyper-cholesterolemia [30,44]. *P. ostreatus* is also nutritionally rich in carbohydrates and vitamins [30-31,45].

In recent years, efforts are being made to cultivate and further improve the nutritional composition of edible mushrooms. Beelman and Lee [46], highlighted that new cultural and post-harvest practices for mushrooms and other organisms may be developed to improve their nutritional compositions and quality. Though naturally grown mushrooms could be nutritionally rich but efforts are being made to fortify them further through artificial cultivation. It is therefore very important to inculcate different methods of cultivation of mushrooms that would possible fortify or improve their nutritional compositions and as well add value to their quality for human and animal health.

The present study keyed into Beelman and Lee`s advice, and evaluated the proximate nutrient potential, phytochemical screening and vitamin B-complex composition of *P. ostreatus* cultivated using different substrate organic supplementation techniques. The different substrate organic supplementations are expected to improve the nutrient value and quality of the cultivated *P. ostreatus* product of the present study.

2. MATERIALS AND METHODS

2.1 Collection of Resource Materials

The fruiting bodies of the macrofungi were obtained from the samples cultivated by substrate organic supplementation technique at the Research Unit Demonstration Farm of the University of Port Harcourt, Rivers State, Nigeria. The substrates were supplemented with avocado seeds (AVOS), whole wheat (WWS) and soyabean+avocadoseed+whole wheat+corn/cob (SAWCS). The products of the substrate organic supplementation techniques were the samples used in the present study. They were dried and stored in sealed containers for till needed for further studies.

2.2 Preparation of *P. ostreatus* Extract

The harvested and dried mushroom samples were cleaned and pulverized using a manual grinder. Ethanol extraction was carried out and the filtrate was concentrated using a rotary evaporator at a temperature of 55°C and the concentrate was subjected to evaporation in a water bath at 55°C to obtain a dark paste which was used for phytochemical studies.

2.3 Determination of Proximate Nutrient Potential

Proximate nutrient potential was determined by comparing the current samples with a 2,000 calories reference diet, for adults and children aged 4 and above [47], it was calculated as follows.

Percentage daily value (%DV)

$$= \frac{\text{Amount of A}}{\text{RDA}} \times \frac{100}{1}$$

Where A = amount of nutrient obtained by proximate analysis. This is the weight of the particular nutrient in a specified quantity of the samples.

RDA= Recommended daily allowance

2.4 Phytochemical Screening

A portion of the ethanol extract of *P. ostreatus* was used for the preliminary qualitative phytochemical screening. The procedures described by Duru et al. [38]; Akubugwo et al. [48]; and Edeoga et al. [49] were employed for testing the presence of the following phyto-chemical constituents: Alkaloids, triterprenoids, steroids, tannins and saponins.

2.5 Determination of B-complex Vitamins

The B-complex vitamins were determined by spectrophotometric methods as described by AOAC [50].

2.6 Statistical Analysis

Data are presented as means and standard deviation of triplicate determinations. Values that are statistically significant in Table 1, were established with least significant difference (LSD) at 5% level.

3. RESULTS

The proximate nutrient potential of AVOS, WWS and SAWCS are shown in Table 1. The three samples had high crude protein and crude fibre nutrient potential, average carbohydrate nutrient potential but low lipid and low energy potential. The crude protein potential of SAWCS was the highest followed by WWS and AVOS had the least. SAWCS had the highest crude lipid potential which significantly increased ($p<0.05$) against crude lipid potential of WWS. Value of crude lipid potential of WWS was insignificant ($p>0.05$) against that of AVOS. The crude fibre potential of SAWCS was the highest and was significantly ($p<0.05$) higher than the crude fibre potential value of WWS. Crude fibre potential value of AVOS was significantly ($p>0.05$) lower than that of WWS. The energy potential of SAWCS and WWS were not significant different ($p>0.05$) but they were significantly ($p<0.05$) higher than the total carbohydrate value of AVOS. The calorific potential value of SAWCS was the highest and significantly ($p>0.05$) increased against the calorific values of AVOS and WWS.

Table 1. Proximate nutrient potential of the fruiting bodies of *P. ostreatus* cultivated with different organic supplements.

	Percentage daily value (%DV)/100 g		
Analyte	**AVOS**	**WWS**	**SAWCS**
Crude protein	31.62±0.02^{c}	34.43±0.30^{b}	35.73±0.03^{a}
Crude lipid	1.48±0.02^{b}	1.67±0.29^{b}	2.06±0.02^{a}
Crude fibre	21.91±0.02^{c}	26.00±0.40^{b}	29.03±0.06^{a}
Total carbohydrate	2.70±0.00^{b}	2.82±0.00^{a}	2.82±0.00^{a}
Calorific Potential	14.54±0.23^{b}	14.54±0.23^{b}	15.47±0.01^{a}

Values are mean ± standard deviations of triplicate determinations. Values of an analyte along the same row having the same letters of alphabet as superscript are not significantly different (p>0.05)
Noted: AVOS=avocado seed supplementation,
WWS= whole wheat supplementation, SAWCS= soyabean + avocado seed + whole wheat+corn/cob supplementation

Table 2. Phytochemical screening of the ethanol extract of *P. ostreatus*

Phytochemical	Status
Alkaloids	+ +
Triterpenoids	+ +
Steroids	+ +
Tannins	_
Saponins	_

Table 3. B-complex vitamin compositions and percentage daily value of the fruiting bodies of *P. ostreatus* cultivated with different organic supplements

Analyte	AVOS	%DV	SAWCS	%DV	WWS	%DV	CV%
Thiamine –B_1	0.90	7.5	1.23	10.3	2.07	17.2	43.2
Riboflavin – B_2	1.60	12.3	0.20	1.5	1.11	8.5	74.8
Niacin-B_3	ND	7.9	ND	6.1	ND	2.2	-
Niacinamide-B_3	12.64	0.004	9.79	0.004	3.47	0.004	54.4
Cyanocobalamin-B_{12}	0.001	-	0.001	-	0.001	-	-

AVOS=avocado seed supplementation, WWS= whole wheat supplementation, SAWCS= soyabean + avocado seed + whole wheat+corn/cob supplementation

Phytochemical screening of the *P. ostreatus* ethanol extract as shown in Table 2, revealed the presence of alkaloids, triterpenoids, and steroids. Tannins and saponins were not detected.

The B-complex vitamin compositions of the three samples of *P. ostreatus* (AVOS, WWS and SAWCS) are shown in Table 3. The samples presented the highest content of niacinamide and least of vitamin B_{12}. The concentrations of niacinamide, vitamins B_2, and B_1 were appreciable in the three samples. However niacin, vitamins B_6, B_7, and B_9 were not detected. AVOS had the highest content of niacinamide (12.64) followed by SAWCS (9.79) and WWS the least (3.47). AVOS also had the highest level of riboflavin (1.60) and SAWCS the least (0.20) while thiamine HCl (vitamin B_1) level was highest in WWS (2.07) followed by SAWCS (1.23) and AVOS the least (0.90).

4. DISCUSSION

The proximate nutrient potential of the fruiting bodies of *P. ostreatus* from WWS, AVOS and SAWCS was investigated in this study. The three samples of mushroom showed high potentials of serving as sources of protein and crude fibre. The nutritional compositions of the supplemented organic substrates may have a resulted in the observed differences in the proximate nutrient potential analyte of the studied samples. Okoroah et al. [51]; Ikewuch et al. [52] also presented high nutrient potentials for *A. wilkensiana* and *T. procumbens* as sources of protein and crude fibre which were increased as a result of drying. SACN [53] reported that increased rate of fibre consumption may help to reduce cases of colon cancer, coronary heart disease, digestive disorders, obesity, high blood pressure and diabetes mellitus.

The phytochemistry of the ethanol extract of the fruiting bodies of *P. ostreatus* cultivated by organic substrate supplementation was investigated in this study. The results of the qualitative phytochemical screening indicate that the fruiting bodies of the macrofungi were rich in alkaloids, triterpenoids and steroids. Tannins and saponins were not detected. The work of Okwulehie and Nosike [54] is slightly in contrast with this result because it reported that *P. Pulmonarius* contains tannins, alkaloids and steroids. The result of this study is in agreement with the report of Deepalakshmi and Mirunalini [55] that crude ethanol extract of *P. ostreatus* on qualitative analysis revealed the presence of alkaloids, steroids and terpenoids. The pharmacological properties of the macrocrofungi may be due to the presence of these phytochemicals. Chang and Buswell [56] indicated that mushroom may possess medicinal properties and the bioactive compounds responsible for these properties can enhance human health quality. Due to the presence of these phytochemicals, *P.ostreatus* may have nutraceutical potentials. Alkaloid such as quinine is used to treat malaria, ephedrine for asthma and homoharringtonine is an anticancer agent [57].

The concentration of riboflavin (B_2) in all the samples analyzed ranged from 0.20-1.60 µg/g. The values reported in the study were comparable to the values (0.08-0.17) reported already by Okwulehie and Odunze [58]. The differences observed in the concentration of vitamins in the mushrooms may be attributed to the nature of substrate, type of organic supplementation and species of mushroom cultivated. Riboflavin is a precursor of flavin mononucleolide (FMN) and flavin adenine dinucleotide (FAD). These derivatives of riboflavin help in the folding of proteins by oxidative process and subsequently its secretion [59]. Vitamin B_2 may help diabetics to manage the case of cardiomayopathy by building the level of antioxidant in their body [59]. The values of thiamine were comparable to that reported by Okwulehie et al. [60] in *Pleurotus* species but higher than the level highlighted by Okwulehie and Odunze [58].

Vitamin content is very important when considering the overall nutritional value of food [61]. Niacinamide was detected in the three samples studied. The value of thiamine HCl was highest in WWS and least in AVOS. The values of thiamine in the samples were appreciable. Vitamin B_{12} had the least value among all the B-complex vitamins analyzed and this record is in agreement with that of Fredrick et al. [62] who got the values of cyanocobalamin to be less than 0.3 µg/100g in their study. Niacin and niacinamide are both precursors of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) in vivo [63]. NAD is important in the catabolism of fat, carbohydrate and protein. It also aids in the repair of DNA as well as cell signaling while NADP is essential in fatty acid production and cholesterol synthesis [64]. Niacin reduces VLDL- and LDL-cholesterol levels and increases HDL-cholesterol in the plasma. This means that it may help to reduce the incidence of cardiovascular diseases [65].

5. CONCLUSIONS

The results in this study therefore show that the three samples of *P. ostreatus* obtained by substrate organic supplementation were rich in crude protein, fibre, vitamin B_1 and phytochemicals such as alkaloids, triterpenoids and steroids. The results suggest that the technique of organic supplementation may improve the nutrient potential and bioactive composition of *P. ostreatus* for their use as food, feed and in medicinal formulations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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