COMPARATIVE PROXIMATE AND QUALITATIVE PHYTOCHEMICAL EVALUATION OF THREE SPECIES OF OYSTER MUSHROOMS CULTIVATED IN NIGERIA

AU Onyechi[1] and MC Afieroho[1,2]

[1] Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka,
[2] Department of Nutrition and Dietetics, University of Nigeria Teaching Hospital, Enugu

Corresponding authors email: Mercyuzoka@yahoo.com

ABSTRACT

Background: Edible mushrooms such as *Pleurotus tuber regium* fruiting body (PTF), *Pleurotuspulmonarius* (PP) and *Pleutotusostreatus* (PO) are valued the world over for their food and medicinal uses.

Objective: The study examined the nutrient composition, and qualitative phytochemical evaluation of three commonly cultivated species of oyster mushrooms: *Pleurotus tuber regium* fruiting body (PTF), *Pleurotuspulmonarius* (PP) and *Pleutotusostreatus* (PO).

Materials and Method: The mushroom was sorted, destalked, washed, dried in a hot air oven at 55ºC - 60ºC for 24 hours and ground into mushroom flour with milling machine and sieved (0.25mm) (1). Flour was packaged in air tight container and kept in refrigerator prior to analysis. The proximate and phytochemical analysis of three selected edible mushrooms (*Pleurotus tuber regium* fruiting body(PTF), *Pleurotuspulmonarius* (PP) and *Pleurotusostreatus*(PO)) flour per 100g were done. The data collected was statistically analyzed using means, standard deviations, analyses of variance (ANOVA) at $p \leq 0.05$ and Duncans new multiple range test.

Result: The result of the nutrient analysis of the three selected cultivated edible mushrooms per 100gram showed that *Pleurotus tuber regium* fruiting body(PTF) had the highest crude fibre (16.38±0.09), ash (9.78±0.19) and moisture (9.56±0.51) content. *Pleutotusostreatus* (PO) had the highest carbohydrate (63.58±0.85) and Fat (1.72±0.19) content, while *Pleurotuspulmonarius* (PP) had the highest protein (11.27±0.44) content. The phytochemical screening of the three (3) mushrooms revealed the presence of alkaloids, glycosides and steroids while flavonoids and tannins were not detected in the mushroom studied.

Conclusion: The three selected cultivated edible mushrooms PTF, PP and PO studied per 100gram showed that they are good sources of nutrients.

Key words: *Mushrooom, Pleurotus species, nutrient composition, phytochemical screening*

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INTRODUCTION

Globally, edible mushrooms are valued for their nutritive and medicinal uses..They have good taste, appetizing aroma, and nutrient contents which is low incalories, high in m**in**erals, essential am**in**o acids, vitam**in**s and fibers (2) Mushrooms have been prescribed for treatment of diseases such as gastro – intestinal disorder, bleeding, high blood pressure and various bacterial infections for centuries (3). Those that are common belong to the following genera:*Lentinus, Garnoderma, Corydiceps, Auricularia, Volvaleria, Termitomyces* and *Pleurotus* (the Oyster mushrooms)(4). The oyster mushrooms like every other edible mushroom, are valued throughout the world as both food and medicine for thousands of years. Their antitumour (5), antioxidant (6), antibacterial properties (7, 8), antidiabetic properties (9), antiviral (10) and nematophagous (11) properties have been reported. The cause of inadequate consumption of mushroom is lack of the knowledge of the nutrient composition of edible mushrooms and their health benefits. Also there is lack of knowledge to identify edible and toxic mushrooms. It has been reported that large numbers of the unknown species of mushrooms whose health promoting properties are unknown reside in Africa and probably in Nigeria. This is because there are little or no data about them (12). The aim of this study is to comparatively evaluate the nutrient composition and qualitative phytochemical composition of the fruiting bodies of three commonly cultivated species of oyster mushrooms: *Pleurotus tuber regium* fruiting body (PTF). *Pleurotuspulmonarius* (PP) and *Pleutotusostreatus* (PO).

MATERIALS AND METHOD

Samples collection

The different species *Pleurotus tuber regium* fruiting body cultivated mushroom was collected from Faculty of Agriculture farm, University of Port Harcourt, Rivers state, *Pleurotuspulmonarius* and *Pleurotusostreatus* of cultivated mushrooms were collected from dilomat farm, Port Harcourt.

Processing of mushrooms flour

The fruiting bodies of the selected oyster mushrooms were sorted, destalked, washed, dried in a hot air oven at 55°C - 60°C for 24 hours and ground into mushroom flour with milling machine and sieved (0.25mm) (1). Flour was packaged in air tight container and kept in refrigerator prior to analysis

Proximate analysis of the mushroom flours

The crude protein, fat, moisture, ash and crude fibre contents of the mushroom flours were determined according to (13).

Determination of carbohydrate

The total carbohydrate was determined by subtracting the percentage (%) of fat (F), % crude protein (Cp), % moisture (M) and % ash content (A) from 100 (14), % Total carbohydrates =100- (F+Cp+M+A)%.

Determination of crude protein

This was determined by a modification of the Kjeidahlgunnings procedure for organic nitrogen. The nitrogen of protein and other compounds were converted to ammonium sulphate by acid digestion with boiling sulphuric acid. A known weight of sample was placed in Kjeldahl flask and about 200 milligram of catalyst mixture was added. 10.0cm^2 of concentrated sulphuric acid was added to the content of the flask. Heat was applied gently for few minutes until frothing ceases and the heat was increased to digest for 3 hours. It was allowed to cool and made to a known volume with distilled water (100cm^3). 10.0cm^3 aliquot of the dilute solution of the digest was distilled by pipetting the volume into distillation chamber of micro Kjeldhal distillation apparatus. 10.0cm^3 of 40% sodium hydroxide solution was added and steam distilled into 10.0cm^3 of 2% boric acid containing mixed indicator (note colour from red-green) titrate with standard 0.01N or 0.2N hydrochloric acid to grey end point.

$$\%\ N\ =\ \frac{(a-b)\ x\ 0.01\ x\ 14.0057\ x\ c}{d\ x\ e}\ x\ 100$$

a = titre value for the sample
b = titre value for the blank
c = Volume to which digest is made up with distilled water
d = Aliquot taken for distillation
c = Weight of dried sample (mg)

Convertion to % crude protein, was multiplied by necessary conversion factor (6.25).

Determination of fat

The method used was that of exhaustive soxhlet extraction using the non-polar organic solvent-petroleum ether (B.p = 40°C to 60°C). A soxhlet extractor with reflux condenser and a small flask which has been previously dried in the oven and weighed were fitted, 2gm of sample was weighed and transferred to a fat-free extraction thimble, it was plugged lightly with cotton wool, the thimble was placed in the extractor and about 150cm^3 of petroleum ether (B.P. 60-80^{0}C) was added into the flask until it siphons over once. More ether was added until the barrel of the 100ml extractor was half full, the condenser was replaced, the joints were tight and placed on the water bath or electrothermal heating mantle. The source of heat was adjusted so that the ether boils gently, it was left to siphon over for at least 8 hours, until the ether was just short of siphoning over, the flask was detached and the contents of the barrel of the extractor was siphoned into the ether stock bottle. It was well drained, and the thimble removed and dried in the oven. The condenser was replaced and distilling the ether continued until the flask was practically dried. The flask was detached (which now contains all the oil), the exterior was cleaned and dried in the oven to constant weight. The extracted residue was kept for the "fibre" determination.

$$Ether\ extracts\ =\ \frac{Wt\ of\ oil}{Wt\ of\ biological\ material}\ x\ 100$$

Moisture

The water content was determined by weighing out 2.5g into silica dish, which has been previously dried and weighed. The dish including the sample inside it was placed in hot air oven for 24 hours at 60^0-70^{0}C (drying at high temperature may result in losses of heat labile or volatile component). It was finally dried to constant weight, cooled for ten minutes in a desiccator each time before weighing.

$$\%\ Moisture\ =\ \frac{W_1 - W_2}{W_1}\ x\ 100$$

W_1 = Weight of biological material before drying
W_2 = Weight of biological material after drying

Ash determination

This was done using the muffle furnace method in accordance with (13). Porcelain crucibles with lids was dried for 15 minutes in a hot air oven at 105°C, cooled in a desiccator and weighed. Two grams of each sample was separately weighed into the appropriately labeled crucible and weighed again. Crucibles and contents were ignited in muffle furnace

at 550°C for 10 hours to light gray ash. Thereafter, sample was removed and placed immediately in a dessicator to cool and the weight was taken. The difference in mass or loss in mass of the crucible and samples before ashing is the organic matter content of each sample. The difference between the mass of the crucibles alone and crucible plus ash gave the mass of ash of each sample. Values for ash was calculated and expressed in percentages.

$$\% \text{ ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of samples}}$$

Determination of crude fibre

Fiber content of the sample was measured using the enzyme modified, neutral detergent fiber (NDF) method. Dried samples whose fat content was extracted using Soxhlet extraction approach was treated with standard NDF procedures up to the point that fiber containing residues was filtered and washed with water. The filtered residues was incubated with a porcine *á* – amylase solution at 37°C overnight. The residues was filtered after incubation, washed very well and dried. The NDF was calculated as filtered residual:

Fibre = residual weight – (weight of protein + ash)

Phytochemical Methods:

Confirmatory phytochemical tests were carried out on the extract using the standard phytochemical screening reagents (15).

Statistical analysis

Data was analysed using statistical product and service solution (SPSS) version 21 to determine the means, standard deviation and standard error of mean (SEM). Analysis of variance (ANOVA) was used to compare the means, Duncan's new multiple range test was used to separate the means. $P \leq 0.05$ was set to be significant.

RESULTS

Table 1 shows the proximate composition of edible mushrooms. The moisture content ranged from 8.67±0.34 to 9.56±0.51. *Pleurotus tuber-regium fruiting* body (PTF) was significantly (P≤0.05) higher in moisture (9.56±0.51) compared to *Pleurotuspulmonarius* (PP) (8.67±0.34) and *Pleurotusostreatus* (PO) (8.82±0.17). Fat content ranged from 0.28±0.19 to 1.72±0.19. PO (1.72±0.19)> PP (0.72±0.19) >PTF (0.28±0.19) which were significantly different (P≤0.05). Ash content ranged from 5.56±0.20 to 9.78±0.19. PTF (9.78±0.19) > PP (6.22±0.51) > PO (5.56±0.20) which were significantly different (P≤0.05) . Protein content ranged from 9.75±0.87 to 11.27±0.44. PP (11.27±0.44) was significantly (P≤0.05) higher compared to PO (9.75±0.87). Crude fibre content ranged from 10.61±0.16 to 16.38±0.09. PTF (16.38±0.09) > PP (12.97±0.14)>PO (10.61±0.16) which were significantly different (P≤0.05). Carbohydrate content ranged from 53.84±0.54 to 63.58±0.85. PO (63.58±0.85)> PP (60.15±1.46)> PTF (53.84±0.54) which were significantly different (P≤0.05).

Table 1: Proximate composition of edible mushrooms per 100g

Nutrients	PTF	PP	PO
Moisture (g)	9.56±0.51	8.67±0.34	8.82±0.17
Fat (g)	0.28±0.19	0.72±0.19	1.72±0.19
Protein (g)	10.17±0.41	11.27±0.44	9.75±0.87
Ash (g)	9.78±0.19	6.22±0.51	5.56±0.20
Crude fibre (g)	16.38±0.09	12.97±0.14	10.61±0.16
Carbohydrate (g)	53.84±0.54	60.15±1.46	63.58±0.85

Mean ± Standard deviation of three determinations.
Key:PTF = *Pleurotus tuber regium fruiting* body, PP = *Pleurotuspulmonarius,* PO = *Pleurotusostreatus*

Table 2 shows the phytochemical screening of edible mushrooms. The phytochemical screening revealed the present of alkaloids, glycosides and steroids and the absence of flavonoids and tannins.

Table 2: Phytochemical screening of edible mushrooms

Nutrients	PTF	PP	PO
Alkaloids	+	+	+
Flavonoids	-	-	-
Glycosides	+	+	+
Tannins	-	-	-
Steroids	+	+	+

Key: + = Positive / Present, - = Negative / Absent, PTF = *Pleurotus tuber regium fruiting* body, PP = *Pleurotuspulmonarius,* PO = *Pleurotusostreatus*

DISCUSSION

Proximate analysis of the mushrooms: The proximate analysis of the three selected cultivated edible mushrooms PTF, PP and PO per 100gram with the work done by Tasnim and Suman (16) who recorded 4.00g for PO flour. The moisture content of the flour was slightly lower than the recommended range (10 to 14%) for flours (17). This is an indication that the mushroom flours obtained and used in this study could be stored without predisposition to microbial contamination and growth. Higher moisture content in flours have been reported to enhance spoilage through creating favourable condition for microbial proliferation as well as enhance enzymatic deterioration (18), since the flours had lower moisture content they are expected to have good shelf life.

Differences were observed between the flours of the mushroom species with respect to their fat content. PO had the highest fat content (1.72±0.19), which was 6.1 times higher than the least (0.28±0.19) found in PTF. This result is not in agreement with the findings of Egwuim*et al.*,(1) who recorded higher (4.80g) fat content of PO. The fat level in mushrooms is almost negligible especially compared with protein and carbohydrate. The fat that is present is mainly the healthy unsaturated fat such as linoleic acid which helps maintain a healthy heart and cardiovascular system (19). The low fat content suggested they would not be good sources of oil.

The protein content of PP flour (11.27±0.44) was generally higher than PTF (10.17±0.41) and PO (9.75±0.87). This value is in contrast with the values therefore be a better source of ash than wheat and plantain.

The crude fiber content varied between the mushroom flours. The fiber content of PTF (16.38±0.09) was 1.5 times higher than the least in PO (10.61±0.16). This result is in agreement with the study by Egwuim*et al.*,(1) who reported that the fibre content of 10 edible species of mushrooms ranged from 3.94 to 20.36g. The fiber contents of the mushroom species was higher than wheat flour (0.27g) reported by Oluwamukomi*et al*., (22), plantain (4.44g) reported by Arisa*et* al.,(21) as well as soy bean (3g) reported by Tasnim and Suman (16). Fibre is reported to have beneficial effects in reducing plasma cholesterol levels in rats (23). The mushroom species in this study is thus a better source of fiber and could therefore be used to fortify low-fiber flours such as wheat in the bakery industry and could be suitable in formulations (24)

The carbohydrate content of the flours of the mushroom species varied from 53.84±0.54 to 63.58±0.85. PO had the highest carbohydrate content (63.58±0.85), which was 1.2 times greater than the least in PTF (53.84±0.54). The carbohydrate content in this result contradicts with the result reported by Egwuim*et al.*, (1)(8.00 to 32.50g). The carbohydrate content of the flours was lower to wheat (68.69g)(Oluwamukomi*et al*.,(22) and plantain (77.43g) (21).

In contrast, the protein, fat and ash contents in this study were lower than the findings reported by Egwuim*et al.*, (1) on 10 edible species of mushrooms, crude fibre contents were similar, while the carbohydrate contents of this study was higher.

Phytochemical screening: The phytochemical screening of the 3 mushrooms revealed the present of alkaloids, glycosides and steroids while flavonoids and tannins were not detected in the mushroom studied. The alkaloids are the class of nitrogenous compounds produced by numerous plants as secondary metabolites for defence, herbivory, and to protect from pathogenic organisms and harmful insects (25). The valuable pharmaceutical properties of mushroom may be attributed to the presence of alkaloids which has been reported to be active against hypertension, arrhythmia, malaria, cancer showed that they are good sources of nutrients. Moisture content (8.67±0.34 to 9.56±0.51) of the mushroom species were similar. This is in contrast reported in previous studies on mushrooms by Egwuim*et al.*, (1) who reported higher protein (14.03 to 60.38g) values. It is also lower than those reported for some protein rich foods such as Soy bean (49.3g) reported by Tasnim and Suman, (12) and mahogany bean *(Afzeliaafricana*) (21.88 – 26.38g) reported by Igbabul*et al.*, (20). However, the protein content of the studied mushrooms were higher than Plantain (*Musa paradisiaca*; 6.04g) reported by Arisa*et al.* (21). The protein content of other species of mushrooms which was found to be in the range of 14.03g - 60.38g (1) is similar to some protein rich foods such as soy bean and mahogany bean from previous studies. The studied mushrooms can therefore be said to be protein rich food and can enhance optimum nutritive value and may therefore be a useful addition for the treatment and prevention of diseases.

The mushroom species had similar ash content, which ranged between 5.56±0.20 and 9.78±0.19. This result contradicts the result of*Egwuim et al.*,(1) who reported higher ash (17.44 to 32.89g). The ash content of mushroom species was higher than wheat (2.78g) reported by oluwamukomi*et al.*(22), plantain (3.08g) reported by Arisa*et al.*,(21), soy bean (2.8g) reported by Tasnim and Suman (16) and mahogany bean (4.78g) reported by Igbabul*et al.*,(20). The high ash content of the flours is indicative that they could be good sources of minerals. The mushroom species would

cardiovascular disorders and human immunodeficiency virus (26). It also acts as topical anaesthetic in ophthalmology, powerful pain reliever, antipuretic action among other uses (27). Glycosides have also been known to lower blood pressure. A similar observation has been made earlier by Xing, Jin, Wang, Tang, Liu *et al.*, (28). The presence of steroids is in agreement with the reported characterization of ergosterol derivatives from Oyster mushrooms species (29).

Conclusion: The three selected cultivated edible mushrooms PTF, PP and PO showed that they are rich in carbohydrate, proteins, fat and crude fibre which are essential in the diet of man. This makes mushrooms to be of high nutritional relevance. The presence of alkaloids, glycosides and steroids which are known phytochemicals with health benefits in these three selected cultivated edible mushrooms PTF, PP and PO studied offer a rationale for their ethnomedicinal uses.

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