COLLECTION, IDENTIFICATION AND CULTURE OF MUSHROOMS IN LAPAI, NIGER STATE, NIGERIA

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Abstract
This study was carried out to collect, identify and culture mushroom under laboratory conditions. The myco- chemical, proximate and elemental analysis of the collected samples of mushrooms *Onniatomentosa*, *Garnodermazonatum*, *Lepiota parasol*, *Plurotus oyster*,*Plurotusoestratus* and *Garnodermaresinacieum*were investigated. Samples were collected from the wild in Lapai, Niger State, and were cleaned to remove any unwanted materials and air dried until they become brittle. One edible mushroom*Plurotusoestratus* was cultured. The result of myco- chemical, proximate and elemental analysis conductedrevealed the presence of Alkaloid and Cyanide in the five Mushrooms samples collected. *Ganodermazonatum*has the highest moisture content 13.89 which was significantly different ($P<0.05$) from others. Ash content and crude fibre of the five Mushrooms were significantly different from each other ($P<0.05$). The fat content which is the oil extract was low with*Lepiota parasols* having 5.48, *Onniatomentosa* (8.16), *Garnodermazonatum* (7.8), *Plurotus oyster* (6.70) and *Gernodermaresinacieum* (6.10). *Plurotus oyster* has the highest content of potassium (2413.50mg/l), while the lowest was recorded in*Ganodermazonatum* with 1640.00mg/l. *Ganodermazonatum* is therefore recommended as alternative to meat since its potassium rate and oil extract was low.

Keywords:Mushroom, Proximate analysis, Elemental analysis, Anti-nutrient,Culturing of mushroom

1.0.Introduction

Mushrooms are primitive organisms known as fungi. The organism lack chlorophyll which synthesize food in higher plants in the presence of sunlight. They do not posses these green colour substances, so they cannot prepare their own food. They grow saprophytically on dead organic matters or other living organisms. Mushroom are fruit bodies or reproductive structures emanating from the mycelium which under natural condition lie buried in the soil or in the substrata where conditions are favourable for their growth. Chang and Miles (1989) define mushroom as a micro fungus with a distinctive fruiting body which can be either epigeous or hypogenous, and large enough to be seen by naked eye and to be picked by hand. Mushroom produce spore that range from white to pink, and shade of yellow to brown to black. Mushroom like Boletes, produce their spores in elongated tubes and the Hedgehog mushroom produce spores on elongated spine.

Mushroom is an edible food accepted by people as daily diet and alternative to meat and fish. It is also a vegetarian diet that gives nutrition of non – vegetable such as some essential amino acids, vitamins and minerals. Nowadays mushroom is being consumed in different ways as curry, snacks, soup, salad, mixed with rice and pickle etc.Mushroom are alsosource of food

for the wildlife. Mushroom is the fleshy, spore bearing fruiting body of a fungus typically produced above ground , soil or on its food source. Mushroom like white button *Agaricusbiosporus can also be cultivated.* To identify mushroom requires a basic understanding of their microscopic structure, most are *Basidiomycetes* and gilled. In most cases, the basidiocarps are highly conspicuous, white to highly coloured, stalked or sensible, above the ground, epiphytic and sometimes underground. Mushrooms have notable place in folklore throughout the world, and the tradition of many culture ancients, past and present. According to Ingold (1973) earlier estimates of described fungi present about 90,000 species, which has been revised recently to a figure around 1.5 million (Monoharacharyee*t al.* 2005), out of which only 50% have been characterised till date, because hardly 10% of them grow artificially. As many as 2000 species of fungi are wildly used for human consumption (Chang and Miles, 2004). However only a handful of them are grown commercially, many are inedible and a few are highly poisonous such as *Ammonitevirosa, Amnitapachycoleg, Lepiotaclypeolariae*e.t.c. (Kurtzman, 1997). The genus *Pleurotus*comprises of over two dozen species most of which are wood rotten saprophytic fungus, a few of family *Ammenceae* (Zervakis*et al.* 2001). The edible fungus *PleurotusOestratus* has been the representative of oyster mushrooms, the most importantly commercially produced species (Eger and Eden, 2003 and Merino*et al.* 2003). Mushrooms are important staple food in the diet of some cultures, usually considered for their flavour, nutritional, medicinal and condiment value. Research conducted suggests mushroom to be a nutritionally sound food that are of greater value to vegetarians. Mattila*et al.* (2001) reported that for thousands of years fructification of higher fungi has been a source of food, and the first species obtained in this way was *Agaricusbiosporus* (Lange) sing. The chemical composition of edible mushroom determines their nutritional value and sensory properties, which differs according to species, and as well varies as per the substratum, atmospheric conditions, age and part of the fructification (Shah *et al.* 1997 and Manzi*et al.* 2001). As a result of their taste, flavour, nutritional value and unique texture, mushrooms are commonly used in home cooking and in catering. According to Chang (1996) mushrooms have been successfully used as appetizers in marinated form, and also as ingredient in soups, sauces, salads, stuffing and meat dishes. Mushrooms also contain many mineral salts and vitamins, particularly of the B and some D groups (Mattila*et al.* 2001). Mushrooms are also rich in mineral constituents, particularly potassium, phosphorus and magnesium with low amount of sodium (Dundar*et al.* 2009).The objective of this study is to collect, identify and culture mushroom in the laboratory of Department of Biological Sciences, Ibrahim BadamasiBabangida University, Lapai, Niger State, Nigeria, and also todeterminethe proximate and myco – chemical composition of the mushrooms collected and identified.

2.0.Materials and Methods

Study Area

Lapai is the headquarter of Lapai Local Government Area of Niger State, adjoining the Federal Capital Territory. Lapai is located on the longitudinal area of 9^0 34 N and latitude 6^0 30 E.It has an area of 3051km^2.Temperature of the area range from 30-37^{0}C yearly, with the highest temperature experienced in the month of March, and with mean total rain fall of approximately 1600mm per annum .

Sample collection

Mushroom species were collected from farm land and environment of the following sampling stations at Lapai:-Cheche, Ibrahim BadamasiBabangida University (Main Campus), State low cost (Lapai), Student villa (Bosso Road) andBadegi Street (Lapai). The samples werecarefully

uprooted by lifting them up holding the tip but firmly very close to rhizomorph, which carries some soil along with it. This is done to avoid damaging the tissue of the mushroom.

Identification

Collected samples of mushroom were taken to the Department of Biological Sciences of Ibrahim BadamasiBabangida University, Lapai for identification with the aid of reference guide.

Preservation of the samples

The samples were preserved by air dried for some days and stored in a transparent polythene bags that are loosely tightened to allow for proper aeration according to Adedayo (2011).

Culturing of Mushroom

The culturing of the mushroom was done under laboratory condition at Department of Biological Sciences of Ibrahim BadamasiBabangida University, Lapai, Niger State, Nigeria. The culturing medium was prepared using saw dust, rice bran and $CaCO_3$, on the dry weight basis of the substrate and was mixed thoroughly with water. The composition was as follows:- 60% Saw Dust, Rice Brain 30% and $CaCO_3$ 10%, and addition of spawn 10g. The medium was filled into nylon bag. The bag was sealed and autoclave at 30 minutes, and allowed to cool for 24 hours, and spawn was added. The medium was incubated to allow the growth of mushroom for 21 days.

Proximate Analysis

The proximate analysis of the samples for moisture, ash and carbohydrate was determined as described by AOAC (2005). Crude protein, Fibre and Fat content was determined by method of Pearson (1976), while mineral contents were determined by atomic absorption spectrometry, flame photometry and spectrophotometry according to the method of AOAC (2003).The phytochemical analysis for the presence of cyanide and alkaloid was carried out according to the method describe by Harbone (1973). The elemental analysis of Iron, calcium, sodium, and potassium was done using the bulk scientific Atomic Absorbance Spectrophotometer(AAS) Model USA.

Moisture Content Analysis

The samples were air dried for some days and stored in a transparent polythene bags that are loosely tightened to allow for proper aeration according to Adedayo(2011). The Sample containers were dried in a hot - air oven at 105^0C for 30 minutes and weighed. 1.0g of grounded sample of mushrooms were placed in the oven-dried containers and weighed. The mushroom samples in the containers were oven-dried to a constant weight at 105^oC in three hours. After three hours, the samples were allowed to cool in desiccatorsand weighed. The loss in weight after drying for three (3) hours was determined accordingly. % Moisture content of the cooled samples were calculated.

Ash Content Analysis

1.0g of each mushroom sample was weighed from the oven-dried sample from moisture content determination. Crucibles were pre-heated in a muffle furnace to exactly 550^oC, cooled in desiccators and weighed. The oven-dried samples were then transferred into the crucibles, placed in a muffle furnace and the temperature raised to 550^oC. After two hours of uninterrupted heating at 550^oC, the crucibles were removed with tong and transferred into the desiccator for cooling. The cooled crucibles were weighed and the weight of the sample left was determined. The Ash Content was calculated as follows:Ash (%) X 100.

Crude Protein Analysis

2.5g of dried and grounded samples were weighed into the digestion tubes. 15 g Na_2SO_4, 1 g Cu_2SO_4, one or two selenized boiling granules and 25 ml. of Conc. H_2SO_4 was added to thetube. It was digested at about 400 ^{0}C until solution wasalmostcolourless (2 hrs for inorganic material) and then at least a further 30 minutes. It was cooled down, 200 ml. of water was added.100 ml 0.1 N HCl was pipette into a 500 ml conical flask, 1 ml Conway's indicator was added, and the flask placed under the condenser of the distillation apparatus, ensuring that the condenser tip is immersed in the acid solution. (Volume of standardized HCl used in distillation may be varied according to the expected nitrogen content of the sample). To the Kjeldahl tube containing the digested samples, 100 ml of 50% NaOH solution was slowly added down the side of the Kjeldahl tube so that it formed a layer underneath the digestion mixture. The digestion mixture was immediately transferred to the distilling bulb of the distillation apparatus and corked. It was heated until all ammonia passed over into the standard acid. Approximately 150 ml. was collected. Excess standard HCl in distillate was titrated with NaOH standard solution until colour changed from purple to greenindicating end point.

The percentage nitrogen (wet weight basis) was calculated as follows:

% Nitrogen (wet) = (A-B) x 1.4007 x 100 / weight (g) of sample

Where:-

A = Vol. (ml.) Std. HCl x Normality of Std. HCl

B = Vol. (Ml.) Std. NaOH x Normality of Std. NaOH

Calculate nitrogen content on dry basis (when moisture content is known) as follows:

% Nitrogen (dry) = % Nitrogen (wet) x 100 / (100 - % moisture)

Calculate the percentage protein (wet or dry basis) as follows:

% PROTEIN = % nitrogen x 6.25

Where, 6.25 is the protein-nitrogen conversion factor.

Crude Lipid Analysis

2g was weighed from the oven dried sample from moisture content determination into a thimble (W1) and the weight of empty thimble (W0). It was further dried in the oven for 5 h at 100^{0}C. Beakers used for fat determination were dried for about 1 h at 100^{0}C and cooled in a desiccator. The weights were taken and recorded (W2).Thethimble containingthesample was placed in a soxhlet unit connected to a condenser and a heating flask. About 400ml. of petroleum ether was poured into the flask in the extraction unit. The heating mantle was set to 60^{0}C and extraction was carried out for 3 hrs.Thesolvent containing theextractedlipid was poured into the dried beaker and evaporated in a stream of air at room temperature until there was no further weight loss. The beaker and extract was weighed (W3).

% Crude lipid = (W3 – W2) / weight (g) of sample X 100.

Crude Fiber Analysis

1 g was weighed from the oven dried sample from moisture content determination. ==> W1, 1.25% sulphuric acid was added to the 150 ml. notch, after preheating by the hot plate in order to reduce the time required for boiling, 3-5 drops of Octanol was added as antifoam agent.It was boiled for 30 minutes exactly from the onset of boiling, Sulfuric acid was drained off. It was washed three times with 30 ml (crucible filled up to the top) of hot deionized water and drained.150 ml. of preheated potassium hydroxide (KOH) 1.25% and 3-5 drops of antifoam was added. It was boiled for 30 minutes. It was filtered and washed. It was washed again with cold deionized water aimed to cool the crucibles and then washed three times with 25 ml of acetone.The crucibles were dried in an oven at105 °C for an hour or up to constant

weight. It was allowed to cool in a desiccator and then weighed. This weight (W2) represents the crude fiber plus ash content in comparison to initial weight.
Calculate the percentage crude fiber:
% Crude fiber =(W2 – W1) x 100 / W1.

Nitrogen Free Extract (NFE) Analysis
% NFE = 100 - (% CL + % CP + % ash + % CF + % Moisture)
Where: NFE = nitrogen free extract
CL = Crude Lipid
CP = Crude Protein
CF = Crude Fiber

Myco - Chemical Analysis
Myco - Chemical analysis for the presence of Cyanide and Alkaloid was carried out by the method described by Harbone (1973).

Elemental Analysis
The elemental analysis of Iron, Calcium, Sodium and Potassium was determined using the Bulk Scientific Atomic absorbance Spectrophotometer(AAS); Model:AccusysManufacutrer : USA.

Statistical Analysis
The results of the nutrients obtained were analysed using non parametric test such as Mann-Whiteney.

3.0.Results and Discussion

Table 1 shows different types of mushroom species collected from the sampling sites and the activities going on at each of the sampling sites.

Table 1: Mushroom Species collected from different sampling sites in Lapai, Niger State, Nigeria

Mushroom species	Sampling sites	Activities going on in the sites
Plurutus oyster	IBBU (Main campus)	Schooling activity
Lepiota parasol	Student Villa (Bosso road)	Residence/Farming
Onniatomentosa	Badegi (Street)	Residence/Farming
Plurutusoestratus	State low cost	Residence/Farming
Ganodermaresinaceum	Cheche	Residence/Farming
Ganodermazonatum	State low cust	Residence/Farming

Table 2 shows that NFE had the highest value for all the proximate analysis and the lowest was observed in the ash content. *Plurotusoyster* had the highest value of 47.99±0.54 in NFE and the lowest value of 4.19±0.04 in the ash content *Lepiotaparasols* had the highest value of 4.19±0.51 in NFE and the lowest value of 45.42±0.26 in the ash content*Onniatomentosa* had the highest value of 53.90±0.60 in NFE and the lowest value of 3.18±0.58in the ash content*Ganodermaresinaceum* had the highest value of 49.22±0.19 in NFE and the lowest value of 3.42±0.62 in the ash content*Ganodermazonatum* had the highest value of 46.04±0.67 in NFE and the lowest value of 3.89±0.895 in the ash content.

Table2: Proximate Analysis of Mushrooms collected from the sampling sites in Lapai, Niger State, Nigeria

Samples	Moisture (%)	Ash content (%)	Crude protein (%)	Crude fibre (%)	Oil extract (%)	N F E (%)
Plurotus oyster	9.60±0.40^{b}	4.19±0.04^{d}	22.81±0.81^{c}	7.35±0.45^{d}	6.58±0.12^{c}	47.99±0.54^{c}
Lepiota parasols	11.48±0.02^{c}	4.19±0.51^{d}	25.52±0.52^{e}	6.00±0.59^{c}	5.43±0.00^{a}	45.42±0.26^{a}
Onniatomentosa	7.28±0.65^{a}	3.18±0.58^{a}	18.52±0.52^{a}	7.85±0.45^{e}	7.66±0.51^{e}	53.90±0.60^{e}
Ganodermaresinaceum	11.08±0.03^{c}	3.42±0.62^{b}	24.20±0.73^{d}	4.65±0.15^{a}	6.00±0.10^{b}	49.22±0.19^{d}
Ganodermazonatum	13.31±0.59^{d}	3.89±0.895^{c}	22.10±0.65^{b}	5.30±0.10^{b}	7.65±0.15^{d}	46.04±0.67^{b}

Mean with the same superscript along the same column are not significantly different (P□0.05).

Table 3 reveals that potassium had the highest value in all the samples of mushroom analysed and calcium had the lowest value. Mushroom species *Onniatomentosa* recorded the highest value of 2588.50±1.50 for Potassium and the least value recorded in species 164.50±0.50.*Ganodermazonatum* recorded the highest value for Iron and the least value of Iron recorded in the species *Plurotus oyster*.The highest calcium value of 89.50±0.50 was recorded by mushroom species *Ganodermazonatum,* while the least value was recorded in *Lepiota parasols* (1.00±1.00).Elemental analysis of sodium revealed the highest value in*Onniatomentosa* (700.50±0.50), while the least value was recorded in mushroom species *Plurotus oyster* (6.04±1.00).

Table 3: Elemental analysis ofMushrooms collected from the sampling sites in Lapai, Niger State, Nigeria

Sample	Iron	Calcium	Sodium	Potassium
Plurotus oyster	9.95±0.15^{a}	3.50±0.50^{c}	6.04±1.00^{a}	2413.50±1.50^{d}
Lepiota parasols	13.68±0.18^{d}	1.00±1.00^{a}	613.50±1.50^{d}	2364.50±0.50^{c}
Onniatomentosa	12.83±0.63^{c}	6.00±1.00^{d}	700.50±0.50^{e}	2588.50±1.50^{e}
Ganodermaresinaceum	11.73±1.23^{b}	2.75±0.75^{b}	472.50±2.50^{c}	1868.50±1.50^{b}
Ganodermazonatum	24.14±0.15^{e}	89.50±0.50^{e}	37.75±2.75^{b}	164.50±0.50^{a}

Mean with the same superscript along the same column are not significantly different (P□0.05).

Table 4 shows the anti – nutrient analysis of the collected mushroom species. It indicates that the alkaloid had the highest value, while Cyanide had the lowest value, while Cyanide had the lowest value in all the sampled mushrooms. *Onniatormentosa* had the highest value of alkaloid 16.12± 0.22, while the lowest value was recorded in Plurotus oyster 3.55± 0.55. In the analysis of Cyanide, the highest value was recorded in the species *Ganodermaresinaceum*0.09± 0.00, and the least value recorded in the species *Lepiotaparasols* (0.03± 0.03).

Table 4: Anti – nutrient analysis of collected Mushroom species

Mushroom Species	Alkaloid	Cyanide
Plurotus oyster	3.55±0.05[a]	0.06±0.05[c]
Lepiota parasols	5.29±0.08[c]	0.03±0.03[a]
Onniatomentosa	16.12±0.22[e]	0.06±0.00[c]
Ganodermaresinaceum	6.39±0.04[d]	0.09±0.00[d]
Ganodermazonatum	4.17±0.04[b]	0.04±0.00[b]

Mean with the same superscript along the same column are not significantly different (P□0.05).

Result of cultured Mushroom *(Plurotusoestratus)*

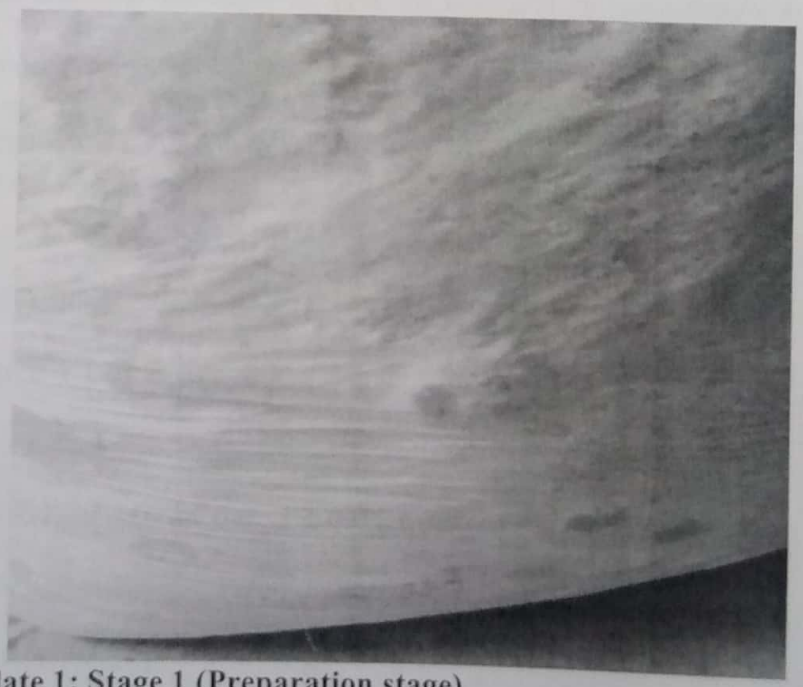

Plate 1: Stage 1 (Preparation stage)

Plate 1 is the preparatory stage which indicates the mushroom cultured with sawdust, rice bran and $CaCo_3$ mixed with spawn, tied in a nylon, autoclave for 30 minutes and allow to cool for 24 hours.

Plate 2: Stage 2 (Mycelium)

Plate 2 shows the appearance of Mycelium on 14th day of the culturing period, as indicated by the arrows.

Plate 3: Stage 3(Fruit bodies)

Plate 3 shows the appearance of fruit body of the mushroom on the 18th day of the culturing period, as indicated by the arrow.

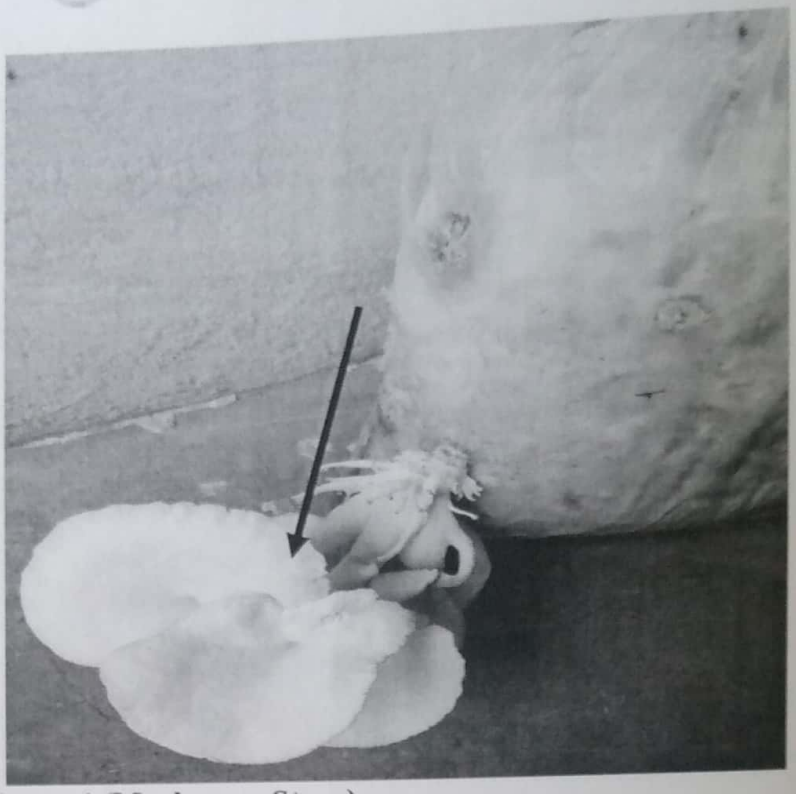

Plate 4: Stage 4 (Mushroom Stage)

Plate 4 indicates the appearance of full grown mushroom on the supplemented substrate, on the 22nd day of the culturing as shown by arrow.

Six species of mushrooms were collected and identified during the study period, from the six sampling sites in Lapai environment; *Plurutus oyster, Lepiota parasol, Omnia tormentosa,Plurutusoestratus, Ganodermaresinaceum and Ganodermazonatum.*The culturing of the mushroom was shown in the plates 1 – 4, and revealed the stages of growth of the mushroom during the cultured period. Plate 1 is the preparation stage, Plate 2showed the emergence of mycelium, plate 3 revealed the emergence of the fruit body of the mushroom, and plate 4 indicates the appearance of the full grown mushroom.The proximate analysis carried on the mushroom collected revealed high moisture contents, which indicates that the mushroom is highly perishable.Although when harvested at full maturity they become tough and almost leathery (Fasidi and Kadiri, 1994).High moisture contents promote susceptibility to microbial growth and enzyme activity.*Lepido parasol* mushroom have the highest protein content followed by *Ganodermaresinaceum,* and *Omnia tormentosa*have the lowest protein content.The relatively high protein contents in these mushrooms is similar to the observations recorded by Fasidi and Kadiri (1994), and is a proof that mushrooms are highly nutritious and good for human consumption(Peter and Tolulope, 2015). The ash content recorded in this study ranged from 3.18g – 4.19g. This differ from the result of Watanabe *et al.* 1994, who recorded ash content range of 5g – 13g per 100g dry matter for edible mushroom.The ash contents of the samples were fairly high indicating its relatively high mineral content (Adedayo, 2011).

The elemental analysis of the five mushrooms recorded in the study revealed the presence of Iron, Calcium and Potassium, which are nutritionally important in considerable and reasonable amount, however Sodium was recorded in minimal amount. This result agreed with the observations of Crisan and Sands (1978) in a similar study. Chang and Miles (2004) also reported that the most common minerals in mushrooms are potassium, phosphorus, sodium, calcium and magnesium. The values were comparable with the values reported from

some wild edible mushroom in Nigeria by Alector (1995). Potassium was found to be the most abundant mineral present in all species of mushroom ranging from 1868.50 – 2588.50 / 100g dry weight. The large amount of Potassium in these mushrooms may be due to the absorption and accumulation of this elements required for metabolic reaction, and regulation of water and salt balance among others.Anti – nutrient screening of these mushrooms revealed the presence of Cyanide and alkaloids, which vary quantitatively from low to highly present. This result is in line with the previous work on mushroom by Kadri and Fasidi.(1992). The presence of alkaloids, according to Peter and Tolulope (2015) indicates that the mushroom may have antibacterial activity, as explained by Idowu (2003) that alkaloids have antibacterial activity. The range of Cyanide recorded in the present study (0.06 – 0.09mg) was much lower than those considered safe by FAO/WHO regulations. A safety limit of 10mg HCN equivalent k^{-1} flour has been fixed by FAO/WHO (1991).

In conclusion, the fat content of mushrooms was observed to be low, which indicates that they can be recommended as alternative to meat, especially for people on low fat dietary food. The study of cultured mushroom show that sawdust and rice bran are potential substrate in mushroom culturing. Also, since the mushrooms are presently collected from the wild, cultivation on a large scale is recommended, and, also investigation of other nutritional parameters should be carried out.

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