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Full Length Research Paper

Food values, heavy metal accumulation, aflatoxin contamination and detection of exo-polysaccharrides in *Lentinus Squarrosulus* Berk, a Nigerian mushroom

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Fruit bodies of a wild edible fungus (*Lentinus squarrosulus*, Berk) were collected from partially buried decaying wood in the swampy area of the Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. They were taken to the laboratory in aerated bags and evaluated for food values, mineral elements composition, heavy metal accumulation, production of exo-polysaccharides and aflatoxin contamination using standard methods. The results showed that fresh samples of this edible fungus had the highest nutrient and mineral element compositions followed in order by 4, 8 and 12 weeks old samples, respectively ($P<0.05$). Protein was the most abundant class of food in the fresh and stored samples. It ranged from 16.87% in the fresh sample and reduced to 15.29% in 12 weeks old samples. Other nutrients detected in significant amount in the fungal samples were ethanol soluble sugars, fat, crude fibre and dried matter. The most abundant mineral element in the fresh samples of *L. squarrosulus* was potassium with value of 12.77 mg/100 g followed in order by phosphorus, magnesium, calcium and sodium ($P\leq0.05$). Traces of heavy metals such as Pb, Cu and Cd were also detected. Aflatoxin G_2 was found to be absent in all the samples. However, 0.005, 0.002 and 0.001 μgKg^{-1} of aflatoxin AFB_1, AFB_2 and AFG_1, respectively were detected in 12 weeks stored samples of *L. squarrosulus*. The vegetative growth of this fungus on basal medium after 15 days showed a mycelial biomass yield of 15.57 mg/ 100 ml while 4.63 units of the exo-polysaccharides were produced. The significance of these observations were discussed.

Key words: Proximate composition, aflatoxin, exo-polysaccharides, *L. squarrosulus*, mineral elements.

INTRODUCTION

Mushrooms including agarics, puffballs and morels are important food supplements in West African countries especially in villages that were exposed to natural vegetation. Edible fungi are used in the preparation of various delicious soup (Jonathan and Esho, 2010). In Nigeria, basidiomycetes such as *Termitomyces microcarpus* (Berk.), *Termitomyces clypeatus* (Heim.), *Termitomyces globulus* (Heim.), *Volvariella volvacea*, (Bull. ex Fr), Singer (*Lentinus subnudus* (Berk.), *Psathyrella atroumbonata* (Pegler.), *Schizophyllum commune* (Fries.) and *Pleurotus tuber-regium* (Mont.), Singer among others, are common species frequently sought for by mushroom hunters (Jonathan, 2002; Fasidi, 1996). This is because they are highly palatable and contain essential food component such as protein, sugars, glycogen, lipids, vitamins, amino acids and crude fibres. They also possessed important mineral nutrients, which are required for normal functioning of the body (Fasidi, 1996; Kuforiji et al., 2003; Gbolagade et al., 2006). Reports on the proximate compositions of edible mushrooms revealed that they are deficient in calories, fats and consist of about 90% water (Manzi et al., 1999; Mushrooms are also rich in vitamins such as nicotinic acid, riboflavin (B_2), thiamine (B_1), vitamin C, vitamin K and trace amounts of vitamin A, D and E (Fasidi and Kadiri, 1991). Parent and Thoen (1977) reported that as

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little as 3 g of fresh mushrooms would provide the recommended daily intake of vitamin B_{12}. The mineral nutrient content in mushrooms is very moderate. Fasidi and Kadiri (1991) reported that potassium and phosphorus are the major mineral elements in *Pleurotus species, L. subnudus* and *Termitomyces robustus*. However, the lipid content of mushrooms has been found to be generally low (Parent and Thoen, 1977; Gbolagade et al., 2006). Mushrooms have high mineral content reported to be almost twice that of any vegetable, while its protein value doubles that of cabbages, potato and six times more than that of orange. Iron, copper, potassium and calcium have also been reported to be available in moderate amount in mushrooms (Zakhary et al., 1983). The crude protein contents of mushrooms are remarkably substantial when compared with those of contemporary foods. Gbolagade et al. (2006) reported that the average protein content (dry weight) of Nigerian mushrooms double that of vegetables and is 4 to 12 times higher than that of fruits. The quality of mushroom protein is valued better than that of cereal grains, legumes, meat, eggs and milk (Jonathan et al., 2009). Ogundana and Fagade (1982) also remarked that Nigerian mushrooms are rich in protein and minerals such as iron, calcium and potassium. Mushrooms are fairly good sources of sugars and crude fibre but poor sources of lipids (Sanmee et al., 2003).

Mushrooms possess very effective mechanisms that enable them to readily absorb heavy metals from their substrates (Lepsova et al., 1988; Turkekul et al., 2004). High concentrations of heavy metals have been observed in the fruiting bodies of mushrooms collected adjacent to heavy metal smelters and oil polluted areas (Kalac et al., 1991; Kalac et al., 1996; Isiloglu et al., 2001).

Edible mushrooms are not usually contaminated by mycotoxins, However, Abu et al. (2009), observed that some stored edible mushroom samples were contaminated by aflatoxins. Likewise, Jonathan and Esho (2010) detected a few aflatoxigenic fungi and aflatoxin contamination in two Nigerian oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus pulmonarius*) stored under natural environmental conditions. Processed and stored Nigerian yam and plantain flours have also been reported to have a significant level of aflatoxins (Jonathan et al., 2011). It has been reported that mycotoxins consumption in foods could lead to cancer or mutation (Borcher et al., 1999)

Therefore, the objectives of this investigation were to determine the mineral nutrients, heavy metals composition, aflatoxin contamination and exopolysaccharide composition in fresh and stored *L. squarrosulus* samples.

MATERIALS AND METHODS

Sample collection

L. squarrosulus samples used in these studies were collected from the partially buried decaying wood in swampy area, within the premises of the Niger Delta University, Wilberforce Island, Bayelsa State Nigeria. Some fruit bodies were analyzed fresh, while some were sundried and stored for 4, 8 and 12 weeks before analyses. These were carried out to know the level of nutrients and possible presence of aflatoxigenic fungi as the storage time increases.

Sample preparation and establishment of mycelial cultures

Tissue culture was carried out on fresh carpophores *of L. squarrosulus* using the method of Jonathan et al. (2009). The mycelia thus generated were cultured on plates of potato dextrose agar (PDA) (Oxoid). The basal medium used consisted of 9.75 g glucose, 3 g yeast extract, 1 g peptone, 0.2 g $MgSO_4 \cdot 7H_2O$, 1 g K_2HPO_4 and 5 g $NH_2(SO_4)$ in 1000 ml of distilled water. *L. squarrosulus* was initially sub-cultured on PDA plates and then 5 mm of the vigorous growing agar plate culture (5 day old) was removed using sterile cork borer. The sterilized basal medium was inoculated with this mycelial disc of *L. squarrosulus*. The flasks were incubated at 28°C for 15 days. All experiments were carried out in triplicate. Mycelial dry weight was also analyzed using the procedure of Jonathan et al. (2009).

Assay for exo-polysaccharides in mycelial culture

Ten milliliters (10.0 ml) of the filtrate was dispensed into a test tube, 3 ml of ethanol, 0.75 ml of phenol and 0.25 ml of 5% H_2SO_4 was added. Standard solution of mycelial culture filtrate of the following concentrations 2.0, 1.8, 1.6., 1.4, 1.2, 1.0, 0.8, 0.6, 0.4, 0.2 and 0.0 (control) were prepared. The absorbance of each was observed using the spectrophotometer at 540 nm (Cecil Instrument CE505). The values obtained were plotted against the concentrations of the standard solution. The 72 hourly concentrations of the exo-polysaccharides were then determined by making extrapolations on the graph against the initial values obtained from spectrophotometer readings of the filtrate (Adebayo-Tayo and Ekerete, 2010).

Extraction, detection and quantification of aflatoxin

The extraction, detection and quantification of aflatoxin were done according to the method of Jonathan and Esho (2010). 5 g of sample was added to 7 ml of distilled water and 25 ml of chloroform, the mixture was shaken and left for 30 min after which the solution obtained was filtered using a Whatman No. 1 filter paper. Extract was obtained and evaporated to dryness to a volume of 5 ml on a hot water bath (Gallenhamp,England) 0.5 ml of the reconstituted extract with chloroform was spotted on a pre-coated 20×20 cm thin layer chromatography (TLC) plate along with aflatoxin standard of known concentration. Developed TLC plate was air-dried at ambient temperature (28±20°C) and aflatoxins were detected under UV light at wavelength of 360 nm (Cecil Instrument CE505). 0.5 µm thick preparative TLC plates were employed for the quantization. On detection of the area containing the toxin of interest, it was scrapped off, elutes with Chloroform and filtered using Whatman No 1. filter paper. The extract was evaporated to dryness and reconstituted with 3 ml Chloroform. Alongside with aflatoxin standard of 20 ug/ml concentration, the absorbance was determined on an ultraviolet spectrophotometer (Cecil Instrument CE505) at a wavelength of 360 nm (Jonathan and Esho, 2010). Aflatoxin concentration in ug/kg was calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{conc. of standard} \times \text{dil. factor}}{\text{Absorbance of standard}}$$

Crude protein determination

The crude protein in the mushroom, was determined by the routine semi-micro kjeldjal procedures. 0.2 g of the each sample was weighed into the Kjeidahl digestion tube. 1 tablets of selenium catalyst and 10 ml of conc. H_2S0_4, was added into the digestion preset at 500°C. The digestion was left for 4 h. The tube was placed in a distilling unit, and 5 ml of 40% NaoH was added to it. The mixture was steam distilled for 2 min into a 50 ml flask containing 10 ml of 44% Boric acid, mixed with indicator solution which was then titrated against 0.1 mHCL until a pink colour was obtained. The quantification was done using AOAC procedure (1990).

Determination of crude fat

Two grammes of each dried sample was weighed into fat free extraction thimble and plug lightly with cotton wool. A soxhlet flask was then filled to ¾ of its volume with petroleum ether and the ether was left on heater to siphon until it was short of siphoning. Ether content of the extractor was drained into the ether stock bottle. The thimble containing sample was then removed and drid on a clock glass on the bench top. The extractor, flask and condenser were replaced and the distillation continued until the flask was practically dry. The flask containing the fat was detached; its exterior cleared and dried to a constant weight in the oven, and the crude fat was determined (AOAC, 1990).

Determination of minerals

0.5 g of the sample was weighed and 10 ml the digested mixture was added, which was prepared in ratio 2:1 of HNO_3 (nitric acid) and percholric acid $HClO_4$ in digested tube. The digestion tube was placed on a digestion block. Digestion took place for 2 h and the solution was allowed to cool in a fume cupboard. The sample was transferred into a 25 ml volumetric flask. The metals were quantified by atomic absorption spectrometry using Cecil Instrument CE505 (Turkekul et al., 2004)

Determination of crude fibre

Two grammes (2.0 g) of the sample was weighed (using chemical balance) into a flask and 100 ml of 0.25 M of H_2SO_4 was added. The mixture was heated for an hour in an oven. The mixture was sieved with a fibre sieve cloth. The residue was returned into the fibre flask and 100 ml of 0.31 NaOH was added and heated for another hour. The mixture was sieved with the fiber cloth. 10 ml of acetone was added. The residue was washed with hot water on the sieve cloth. Then it was transferred into the crucible. The residue and the crucible were oven-dried at 105°C. The crucible containing the residue was cooled in a desiccator and later weighed to obtain the weight. The crucible with the residue was transferred to the furnace to ach at 550°C for 4 h. The crucible containing the ash was cooled and weighed to obtain W_2. W_1 to W_2 gives the weight of the fibre (AOAC, 1990):

$$\% \text{ Fibre} = \frac{(W1\text{-}W2)\ 100}{\text{Weight of sample}}$$

Determination of ash

Empty crucible was weighed and 2.0 g of the sample was weighed into the crumble and transferred into a furnace at 550°C for 4 h. The crucible and the ash was cooled in a desiccator and weighed:

$$\% \text{ Ash} = \frac{a \times 100}{\text{Weighed of sample}}$$

Determination of moisture content

The weight of the empty crumble (W_0) was determined. 2.0 g (W_1) of the sample was weighed into the crucible. The sample was placed in the oven 100°C for 2 h. The sample was cooled in a desiccator for 10 min. Weight of the oven dried sample (W_3) was determined:

$$\% \text{ Dry matter} = \frac{W_3 - W_0\,(100)}{W_1 - W_0}$$

% Moisture = 100 - %DM

Ethanol soluble sugars

These were carried out using the method of Fasidi and Kadiri (1991).

RESULTS AND DISCUSSION

The results of the proximate analyses of the harvested wild mushroom samples were presented on Table 1. The percentage values obtained for the crude protein for fresh (0 day), 4, 8 and 12 weeks old samples were (16.87±0.08, 16.79±0.04, 15.51±0.04, 15.29±0.02), crude fat (0.22±0.00, 0.17±0.01, 0.13±0.01, 0.11±0.01), crude fibre (6.73±0.02, 6.62±0.02, 6.59±0.02, 6.41±0.01), ash (2.31±0.01, 2.40±0.01, 2.36±0.01, 2.17±0.01), dry matter (7.64±0.01, 91.22±0.02, 89.09±0.02, 88.76±0.05), moisture content (92.36±0.01, 8.78±0.02, 10.91±0.02, 11.24±0.02) and % of ethanol soluble sugars (6.88±0.02, 6.84±0.05, 6.76±0.05, 6.40±0.04, 64.40±0.02). These results showed that *L. squarrosulus* is rich in essential food components. This observation concurred with the report of Fasidi (1996) on *Volvariella esculenta* (Mass), Fries.

The values obtained for the crude protein, crude fat, crude fibre, ash, dry matter, moisture content and ethanol soluble sugars were all significantly different at $P \leq 0.05$ for all the treatments. However, it was observed that the nutrients in the mushroom fruit bodies decrease with rising incubation period because of microorganisms which uses the available nutrients for their growth and metabolism. The nutrient constituents of mushrooms have been reported to be affected by the mushroom type, part of the fruit bodies under study and nature of substrate utilization (Fasidi and Kadiri, 1991; Fasidi, 1996, Gbolagade et al., 2006). This may explain the reduction trend in the parameters analyzed (proximate and mineral element compositions). Table 1 showed that protein was the most abundant class of food nutrient in the fresh and stored samples. It ranged from 16.87% in the fresh sample to 15.29% in 12 weeks old samples. This result is

Table 1. Proximate composition of *L. squarrosulus* at different time of storage.

Parameter	Mean of 3 replicates (in %) ± SD			
	A	B	C	D
Moisture	92.36^{a}±0.01	8.78^{d}±0.02	10.91^{c}±0.02	11.24^{b}±0.05
Dry matter	7.64^{d}±0.01	91.22±0.02^{a}	89.09^{b}±0.02	88.76^{c}±0.05
Crude protein	16.87^{a}±0.08	16.79^{b}±0.04	15.51^{c}±0.04	15.29^{d}±0.02
Crude fat	22^{a}±0.02	0.17^{b}±0.01	0.13^{c}±0.01	0.11^{d}±0.01
Crude fibre	6.73^{a}±0.02	6.62^{b}±0.02	6.59^{c}±0.01	6.41^{d}±0.02
Ash	2.31^{c}±0.01	2.40^{d}±0.01	2.36^{b}±0.01	2.17^{a}±0.01
Sugars content	6.88^{a}±0.02	6.84^{a}±0.05	6.76^{b}±0.04	6.40^{c}±0.02

A, fresh (a day old); B, 4 weeks old; C, 8 weeks old; D, 12 weeks old; Values are means of 3 replicates. Means followed by same letter along each column are not significantly different by Duncan's multiple range test (P≤0.05).

Table 2. Mineral elements analyses of *L. sqarrosulus* at different time of storage.

Variable	Mean ± SD (mg/g)			
	A	B	C	D
Ca	1.78^{a}±0.01	1.56^{b}±0.02	0.88^{c}±0.01	0.43^{d}0.01
Mg	2.94^{a}±0.01	2.01^{b}±0.01	1.90^{cd}±0.01	1.50^{d}±0.01
K	12.77^{a}±0.01	11.72^{b}±0.03	10.60^{c}±0.01	10.53^{c}±0.02
P	6.22^{a}±0.01	5.49^{b}±0.02	5.24^{c}±0.02	4.53^{d}±0.02
Zn	0.32^{a}±0.33	0.260^{b}±0.40	0.082^{c}±1.03	0.058^{d}±1.53
Na	1.86^{a}.50	1.58^{b}±0.02	1.50^{bc}.±0.58	1.45^{c}±0.010
Mn	0.055^{a}±1.04	0.024^{b}±0.55	0.019^{c}±1.40	0.016^{d}±1.32
Fe	0.90^{a}±5.77	0.88^{b}±1.75	0.52^{c}.±5.00	0.039^{d}±1.58

A, fresh (a day old); B, 4 weeks old; C, 8 weeks old; D, 12 weeks old; Values are means of 3 replicates. Means followed by same letter along each column are not significantly different by Duncan's multiple range test (P≤0.05).

in line with that of other workers (Suzuki and Oshima, 1976; Ogundana and Fagade, 1982; Gbolagade et al., 2006).

Table 2 showed the mineral element composition of samples. *L. squarrosulus* potassium was the most abundant mineral element in the fresh and stored samples. Its value ranged from 12.77 to 10.53 mg/g in fresh (0 day) and 12 weeks samples respectively. This was followed in order by phosphorus, magnesium, sodium and calcium (P≤0.05). The values obtained for zinc and iron were relatively low. This present results contradict the observation of Zakhary et al. (1983) who recorded magnesium and iron as the most abundant mineral elements in wild mushrooms collected from Egypt. The difference in results may be due to variation in environmental factors, collection points and/or habitat and nature of mushroom used. Ogundana and Fagade (1982) also found out that mushrooms are good source of mineral elements such as iron, calcium and potassium.

The heavy metal level in wild harvested *L. squarrosulus,* that is, Cu, Pb, Cd and Cr were significantly high (P≥0.05) in both fresh and stored samples used in this study (Table 3). The level of these metals recorded in *L. squarrosulus* may be linked to the site of collection. This mushroom could have accumulated these metals from different wastes dump in which it was growing. Mushrooms have been reported to possess very effective mechanisms that enable them to readily absorb heavy metals from their substrates (Cibulka et al., 1996; Isiloglu et al., 2001; Turkekul et al., 2004). High concentrations of heavy metals have been observed in the fruiting bodies of mushrooms collected adjacent to heavy metal smelters and oil polluted areas (Kalac et al., 1991, 1996; Isiloglu et al., 2001).

The quantity of aflatoxin AFB_1 and AFB_2 detected in 12 weeks old mushroom samples were 0.005 and 0.02 μgKg^{-1} respectively while 0.001 μgKg^{-1} of AFG_1 was detected in 12 weeks fungal sample (Table 4). These values were very low and cannot cause serious health hazards for consumers (Adebayo-Tayo and Ekerete, 2010). However, Jonathan and Esho (2010) reported higher level of $AFB_1$and AFB_2 in *P. ostreatus* and *P. pulmonarius* stored for 105 and 140 days under Nigeria natural storage condition The aflatoxin level permitted in

Table 3. Heavy metal composition of *L. squarrosulus* at different time of storage.

Variable	Mean±SD (mg/100 g)			
	A	B	C	D
Cu	0.69[a]±0.05	0.56[b]±0.25	0.50[c]±0.20	0.42[d]±0.12
Pb	050[a]±0.50	040[b]±0.76	032[c]±0.58	028[d]±0.58
Cr	0068[a]±0.08	0063[b]±0.15	0059[c]±0.25	0050[d]±0.85
Co	0.10[a]±0.00	0.087[b]±0.06	0.073[c]±0.06	0.064[d]±0.06
Cd	0027[a]±0.06	0027[a]±0.15	0025[a]±.05	0.0024 [a]±0.06

A, fresh (a day old); B, 4 weeks old; C, 8 weeks old; D, 12 weeks old; Values are means of 3 replicates. Means followed by same letter along each column are not significantly different by Duncan's multiple range test (P≤0.05).

Table 4. Aflatoxin analysis of *L. squarrosulus* at different time of storage.

Variable	Mean ± SD (µg/100 g)			
	A	B	C	D
Aflatoxin B1	0.00[c]±0.00	0.00[c]±0.00	0.00[b]±0.00	0.005[a]±0.00
Aflatoxin B2	0.00[b]±0.00	0.00[b]±0.00	0.00[b]±0.00	0.002[a]±0.00
Aflatoxin G1	0.00[b]±0.00	0.00[b]±0.00	0.00[b]±0.00	0.001[a]±0.00
Aflatoxin G2	0.00[a]±0.00	0.00[a]±0.00	0.00[a]±0.00	0.00[a]±0.00

A, fresh (a day old); B, 4 weeks old; C, 8 weeks old; D, 12 weeks old; Values are means of 3 replicates. Means followed by same letter along each column are not significantly different by Duncan's multiple range test (P≤0.05).

Table 5. The mycelial biomass yield and exo-polysaccharide production of *L. squarrosulus* grown in submerged liquid medium.

Day	Mycelial yield (mg/ 100 ml)	Filtrate absorbance (540 nm)	Exo-polysaccharides (Mg/ 100 ml)
1	0.64[d]	0.88[d]	0.35[c]
3	1.83[d]	0.98[d]	0.88[c]
6	7.26[c]	1.26[c]	1.50[b]
9	9.83[b]	1.41[b]	2.61[a]
12	10.95[b]	1.45[b]	3.22[a]
15	14.57[a]	2.68[a]	4.63[a]

Values are means of 3 replicates. Means followed by the same letter(s) is not significantly different by Duncan's multiple range test (P≤ 0.05).

Nigerian food is 20 µg/kg (Bankole et al., 2004). However, Jonathan et al. (2011) reported that the longer the storage period, the higher the chance of aflatoxins production. This may be due to higher fungi activities in stored food products.

Table 5 showed the results of the mycelial biomass growth and exopolysaccharride produced by *L. squarrosulus* when grown in submerged liquid medium. After 15 days of fermentation, the mycelial biomas recorded was 14.57 g/ 100 ml while 4.63 units of the exopolysaccharride were produced. Many workers have isolated polysaccharides from different mushrooms e.g. Kim et al. (2006) for *Phellinus linteus* and Leung et al. (1997) for *Flammulina velutipes*. The major bioactive compounds found in mushrooms are the polysaccharides. Many polysaccharides isolated from mushrooms are considered to act as biological response modifiers (BRM) and enhance various immune responses (Kim et al., 2006). BRMs are substances that stimulate the body's response to infection and disease. Unlike proteins and nucleic acids, they contain repetitive structural features that are polymers of monosaccharides residues joined to each other by glycosidic linkage (Sharon and Lis, 1993). Consequently, they offer a high capacity for carrying biological information because of their structural variability (Wasser, 2002). In another study, Mondal et al. (2006) reported splenocytes activation of rats by polysaccharides isolated from aqueous extract of

Termitomyces striatus. Oyetayo (2007) also observed that *Termitomyces clypeatus (Heim)* input authority has appreciable level of exo-polysaccharides.

Conclusion

It was observed from these studies that fresh fruitbodies of *Lentinus squarrosulus* are rich in protein, sugars and mineral elements. The carpophores of this fungus could accumulate heavy metal if grown in heavy metal polluted environment. The contamination of mushrooms in storage by aflatoxigenic fungi if stored for three months is insignificant to cause health hazards for humans. It is recommended that fresh mushrooms should be eating by mushroom lovers; alternatively health friendly preservatives should be used on stored mushrooms to prevent possible fungal and aflatoxin contamination

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