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REVIEW ARTICLE

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Proximate and Bioactive Compounds Evaluation of Young and Mature Fruit Bodies of *Lentinus Squarrosulus* (Mont.) Sing. Collected from two Log Substrates in Umudike, Abia State Nigeria

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ABSTRACT

Young and mature fruits bodies of Lentinus squarrosulus were collected from logs of Dacryodes edulis and Mangifera indiica in Umudike, Abia State Nigeria. Fresh fruit bodies were separated according to their age (young and mature) and then substrates. Dry powdery samples (DPS) were used to determine their prooximate and bioactive compounds concentrations. Data collected were analysed using Analysis of Variance (ANOVA), while mean separation was done using Duncan Multiple Range Test (DMRT). Results on proximate analysis showed that mature fruit bodies of L. squarrosulus collected from D. edulis (60.18) and M. indica (54.17) gave higher CHO contents, compared to young fruit bodies; while Protein was higher in young fruit bodies collected from D. edulis (18.27) and M. indica (28.40). Bioactive compound concentrations at both stages of the fruit bodies; with respect to their log substrates were not significantly different (p>0.05). Therefore, consumption of L. Squarrosulus; whether young or at maturity, should be of choice depending on the nutrient being sought.

Key words: Lentinus squarrosulus, Mangifera indica, Proximate, Bioactive compounds and Umudike.

INTRODUCTION

Mushrooms grow widely in the tropical and sub-tropical rainforest (Chirinang and Intarapichet, 2009). They are capable of degrading lignin and cellulose using hydrolytic extra-cellular enzymes (Okwujiako *et al.*, 2013). Mushrooms are found growing naturally on different types of woody and non-woody agricultural wastes; including dead plants (Stamets, 2003). Shiitake and Oyster mushrooms have traditionally been produced using the outdoor log technique, although controlled techniques such as indoor tray-growing or artificial logs made of compressed substrate have been substituted (Davis, 2001). Kadiri and Aizai (2005) showed that *Lentinus subnudus* could be cultivated on wood logs of tropical trees. According to Hyunjong and Seung (2004), hard woods such as poplar, willow, beech, elm and alder are the most commonly used tree species in oyster mushrooms cultivation. They noted that unlike Shiitake, Oyster mushrooms do not grow well on oak tree logs. Growing Shiitake on wood logs are still quite popular in Japan, but in most other countries, plastic bag cultivation using sawdust waste is preferred due to lack of suitable wood logs.

In developing countries cultivation of Shiitake on wood logs is a marginal business because of high labour cost (Oei, 2003) *L. squarrosulus* is an edible mushroom commonly found in the wild and has not been cultivated on a large scale for the production of fruit bodies. The tough fruit body is rich in proteins, sugars, lipids, amino acids, vitamins B, C and D; including minerals (Royce *et al*., 1990). Okwulehie and Odunze (2004) reported that *Auricularia aricula-judae, L. squarrosulus* and *Russula sp.* were found to contain appreciable amounts of Alkaloids, Phenols, Saponins, and Flavonoids. Hyunjong and Seung (2004) maintained that since mushrooms feed primarily on sapwood, any tree trunks selected for inoculation should have a larger sapwood area. The lighter or outermost wood of a log is the sapwood and the darker or inner wood is the heartwood. They also reported that a log with a small amount of sapwood would probably produce mushrooms for fewer years than another log with a greater amount of sapwood.

The aim of this research was to determine the appropriate stage to harvest *L. squarrosulus* fruit bodies for better nutritional requirements.

MATERIALS AND METHODS

Fresh fruit bodies of *L. squarrosulus* were harvested from decomposing *Dacryodes edulis* and *Mangifera indica* log substrates, age of mushroom fruit bodies were determined by monitoring primordial initiation till desired stage and by measuring from 0 – 3cm and 3.1 – 8cm using metre rule. The former were considered as young fruit bodies while the later as mature; following the methods of Kadiri (2004), Ayodele and Okhuoya (2009).

Mushrooms were separated according to their source of collection (substrate) and then sizes. The fruit bodies were dried and milled into powder using laboratory manual grinding machine (Oyetayo and Ariyo, 2013).

PROXIMATE ANALYSIS

The ash content was determined by inserting 3g of powdered sample in a furnace at 550^0c for 6 hrs according to AOAC (2000). Moisture contents was determined by placing 2g of the powdered dry samples on a clean dry glass petri dishes of known weight and placed in an electric oven at 75^oc for 7-8hrs (AOAC, 2000 and Konuk *et al.,* 2006). The oven-dried samples were maintained at constant weight (Mukiibi, 1973). Fat content was estimated following the method of AOAC (1980; 2000). 2g of each sample was inserted into an Esther extracting thimble and placed on the soxhlet reflex flask channeled into a round bottom

flask of unknown weight. The apparatus was filled with 250ml of petroleum ether and placed on a heating mantle. Fat content of each sample was determined by the Gravimetric method. While Carbohydrate contents were determined by difference. Lastly, Protein and crude fibre contents were determined by the method of AOAC (1984).

DETERMINATION OF PERCENTAGE BIOACTIVE COMPOUNDS

The percentage flavonoids and saponins were determined following the methods of Bohn and Kloupai-Abyazani (1994) and Peng and Kobayashi (1995) respectively. The percentages were calculated as in Alkaloids

Determination of Phenols

To determine percentage Phenols in the samples, the method of Harbone (1988) was followed. 2g of the sample was used. The absorbance of the solution was read of at 505nm wavelength using a spectrophotometer.

Determination of Tannins

Tannins were determined according to the method of Okeke and Elekwa (2002). 0.5g of the sample in 10ml of 2m HCl was shaken for 5mins and transferred into a volumetric flask and made up to 50ml. The mixture was filtered and 5ml of the filtrate was introduced into a test tube. 3ml of 0.1 NHCl and 3ml of 0.008m of potassium ferro-cynide ($K_3F[CN]_3$) were added. The absorbance was read at 720nm within 10mins.

Statistical analysis

The data obtained were statistically analyzed using Analysis of Variance (ANOVA). Mean separation and tests of significance were carried out by Duncan Multiple Range Test (DMRT) at $p \leq 0.05$ (Steel and Torie, 1980).

RESULTS AND DISCUSSION

Table 1. Effect of Log Substrates and age on Proximate Composition (%) of *L. Squarrosulus* Fruit Bodies.

Log substrate/cap size (cm)	Fat	Crude fibre	Ash	M.C	CHO	Protein
0 – 30cm(young)						
D. edulis	2.17^a	5.94^b	3.80^b	10.16^a	59.52^a	18.27^a
M. indica	2.34^a	4.96^b	4.67^b	10.67^a	49.51^b	28.40^a
3.1 – 8cm (mature)						
D. edulis	2.16^b	6.32^a	4.67^a	10.67^b	60.18^a	16.31^a
M. indica	2.22^b	6.54^a	5.28^a	9.88^b	54.17^a	24.57^a

Values are means of 3 replicates and values bearing the same letter are not significantly different (p>0.05) MC = Moisture Content, CHO = Carbohydrate.

Table I shows the proximate composition of *L. squarrosulus* fruit bodies as affected by their log substrates and age. Fat composition of both young and mature fruit bodies of *L. squarrosulus* harvested from *D. edulis* and *M. indica* log substrates was not significantly different. Mature fruit bodies gave almost equal fat concentrations across *D. edulis* (2.16%) against the 2.22% fat recorded in fruit bodies from *M. indica.* These values were slightly higher than those reported by Okwulehie *et al* (2007) in an experiment comparing pharmaceutical and nutritional composition of some wild macro-fungi found in Nigeria; but

relative to those obtained by Onyeizu *et al* (2017) in *P. pulmonarius* grown on some wood logs. Crude fibre composition of the fungus was lower in young fruit bodies from *D. edulis* (5.94%) and *M. indica* (4.96%); but higher at mature stage at *D. edulis* (6.32) and *M. indica* (6.22%). These are contrary to the results obtained by Garacha *et al* (1993) and kurtzman (1993). Results of percentage ash composition showed that young fruit bodies gave 3.80% and 4.67% with respect to *D. edulis* and *M. indica* respectively. At maturity, fruit bodies from *M. indica* maintained the highest (5.28%) ash concentration than those obtained from *D. edulis* (4.96%) log substrate. Moisture contents of young and mature fruit bodies from both *D. edulis* and *M. indica* was low. In comparison, young fruit bodies from *D. edulis* logs were recorded at 10.14%. This value was relatively higher, compared to moisture content at maturity where *D. edulis* was found to be 10.07% and *M. indica* (9.88%). The above values are lower than the results reported by Okwuliehie *et al* (2007), Ayodele and Okhuoya (2009) as well as Sharad (2013). This could be the reason for the toughness of the mushroom fruit bodies; especially at maturity (Royce *et al,* 1990). CHO composition was lower in young fruit bodies than mature ones. For instance, young mushrooms from *D. edulis* logs gave CHO composition of 59.52% while those from *M. indica* gave 49.51%. CHO composition of *L. squarrosulus* fruit bodies showed significant increase at maturity than at their younger stage. Fruit bodies from *D. edulis* gave 60.18% CHO contents while those from *M. indica* gave 54.52%. These observations were supported by the works of Okwulehie *et al* (2007) and Sharad (2013) who reported high composition of carbohydrate in mature oyster mushrooms.

Apart from the fact that fruit bodies from *M. indica* had higher composition of protein than those of *D. edulis* at all stages of development, protein content of young fruit bodies of *L. squarrosulus* collected from *D. edulis* (18.27%) and *M. indica* (28.40%) log substrates were higher compared to values obtained at maturity. The appreciable amounts of CF, protein and CHO in fruit bodies as generally observed in this study has been attributed to the nature of substrate and to a large extent, mushroom species (Nwoko *et al*., 2006). This further substantiates the claims by Obodai (2003), Adejoye and Fasidi (2009) and Okoi and Iboh (2015) which in separate experiments noted that the nutritional composition of mushrooms could reflect the chemical composition of their substrates; since they carry out extra-cellular digestion of the decomposed substrate during cultivation.

Table 2. Effect of Log Substrate on Bioactive Contents (%) *L. squarrosulus* Fruit Bodies With Respect to Substrates and Age.

Log substrate/cap size	Saponins	Alkaloids	Flavonoids	Tannins
0 – 3.0cm (young)				
D. edulis	1.12^{b}	0.25^{b}	1.92^{b}	0.12^{b}
M .indica	1.44^{b}	0.33^{b}	1.67^{b}	0.12^{b}
3.1–8.0cm (mature)				
D. edulis	1.87^{a}	0.32^{a}	1.84^{a}	0.16^{a}
M. indica	1.97^{a}	0.47^{a}	1.82^{a}	0.16^{a}

Values are means of 3 replicates and values bearing the same letter are not significantly different (p>0.05).

Results on **Table 2** shows that all the bioactive compounds (Saponins, Akaloids, Flavonoids and Tannins) analysed were present in varied concentrations in both young and mature fruit

body samples with respect to their substrates. This could be due to age of the fruit bodies (Garsha *et al.,* 1993). For instance, young *L. squarrosulus* fruit boidies collected from *D. edulis* and *M. indica* subtrates gave 1.12% and 1.44% concentration of Saponins respectively, but gave 1.87% and 1.97 in those of *D. edulis* and *M. indica* respectively. Alkaloids composition were 0.25% and 0.33 in young fruit bodies obtained from *D. edulis* and *M. indica* respectively, while at maturity, the fruit bodies gave 0.32% with respect to *D. edulis* and 0.47% alkaloids. Young fruit bodies from *M. indica* logs had flavonoids content of 1.67% but showed no significant different at maturity (1.82%). Young fruits bodies from *D. edulis* and *M. indica* had tannins concentrations of 1.13% and 0.12% respectively while at maturity; fruit bodies gave equal concentration of Tannins (0.16%). These values were higher than those reported by Onyezu *et al* (2017) and Nwoko *et al* (2017), except in alkaloids they reported concentrations higher than those observed in fruit bodies from both substrates as well as stages of fruit body development.

Edeoga and Erieta (2001) noted that the considerable Pharmacological activities of mushrooms make them to be of high interest in Pharmaceutical industries for the development of drugs. In other words, most bioactive compounds which play essential role in human and animal Physiology have been successfully identified in the studied mushroom.

CONCLUSION

Successfully conducted experiment showed that crude fibre and protein contents of fruit bodies were higher at maturity than younger stage of development; unlike CHO where higher concentrations were recorded at maturity. Apart from age of fruit body, substrate had a significant effect on both proximate and bioactive compounds composition of fruit bodies. It is therefore pertinent to note that consumption of *L. squarrosulus* fruit bodies at any stage of its development should be by choice; depending on the nutrient required by the consumer.

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