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Mycomeat Production through the Solid State Fermentation of Soymilk Waste by *Lentinus Subnudus*

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Abstract
Lentinus subnudus was cultured on soy milk waste with the view of using it to produce edible mycomeat. The solid state fermentation of soymilk waste was done singly without any additive. The basal substrate was then variously constituted with 5 g/l each of sucrose, citric acid and yeast extract. The set-ups were incubated in the dark and monitored daily until full ramification was obtained. The best mycomeat obtained within the shortest time frame was oven dried at 60^{0}C and then blended to powder. The pH, proximate and mineral composition of the mycomeat was determined. *L. subnudus* grown on soymilk wastes took only 11 days in which the resultant mycomeat of *L. subnudus* was found to be meaty-like in appearance and aroma. Oven-dried samples became coarse in texture with a pH of 3.91. Proximate analysis of the mycomeat showed 2.13% moisture content, 14.44% crude protein, 51.8% carbohydrate 9.56% crude fibre, 15.82% ether extract and 5.75% ash content. The percentage mineral composition revealed it to contain; Ca^{2+} (0.62±0.02), Mg^{2+} (0.03±0.01), K^+ (0.06±0.01) Na^+ (15.90±0.30), Mn^{2+} (8.20±0.04), Fe^{2+} (19.22±0.21), CU^{2+} (2.74±0.02) , Zn^{2+} (7.17±0.01) Mo^{2+} (0.05±0.01) and Vitamin C (0.24±0.01). The resultant mycomeat represents a viable source of nutraceuticals .
Keywords: Mycomeat, *Lentinus subnudus*, soymilk waste, mineral composition, proximate analysis.

1. Introduction

Several fermented foods have been around times immemorial and are still available all over the universe; tempeh, a Chinese delicacy is produced through the fermentation of soybeans with Aspergillus oryzae. Yoghurt is produced by the fermentation of milk with different Lactobacillus species and 'Ogiri' is produced from fermentation of melon seed (*Citrullus vulgaris*) in the western part of Nigeria (Odunfa and Oyewole, 1995). Fermented foods are known for their good nutritional and health benefits, and are often produced via solid state fermentation (SSF) technology, a process in which micro-organisms are grown on solid substrates in the absence of free water (Lagemaat and Pyle 2001). Chang and Miles (1989), coined "mycomeat" to refer to fungal protein obtained through the conversion of food processing biomass wastes; most times via SSF. Edible fungi, mainly mushrooms can be cultured for their fruiting body, metabolites such as enzyme, or mycomeat (containing both the growth substrate and the mycelial of the fermenting fungi). Okwulehie and Ogoke, (2013) reported that mushrooms are good sources of proximate components and minerals needed for good health. In Nigeria villages, certain mushrooms such as *Termitomyces robustus, T. globulus, Volvariella esculenta, V. volvacea, Lentinus subnudus* and young sporophores of *P. tuber-regium* are usually served as alternative to meat (Jonathan and Fasidi 2005). *L. subnudus (*also referred as *L. squarrosulus*) is one of the most common Nigerian edible mushrooms. This highly prized fungus usually grows wildly on decaying wood during the raining season (April-October). It could be easily identified by the tough mixture of matured sporophores, velvety stipe and funnel-shaped whitish pileus (Jonathan 2002).

L. subnudus could be chewed for a long time and because of its meaty taste serves as a good alternative to animal protein among rural dwellers and average Nigerians who cannot afford the high cost of meat, due to the daily rise in the cost of living. It has been reported to be a very good source of amino acids, glycogen, sugar, lipid, ascorbic acid and vitamins (Kumari et al., 2011). In Nigeria, *L. subnudus* and other edible mushrooms are important dietary components. Mushrooms are valuable health foods that is low in calories, high in vegetable proteins, chitin, iron, zinc, fibre, essential amino acids, vitamins, and minerals, such as copper that help the body to produce red blood cells (Aina et al., 2012). Creation of an array of food varieties has long plagued mankind so as to prevent the boredom experienced in eating same or related food varieties. Mushrooms are often regarded as highly nutritious and having many health benefits (Kumari et al., 2011); however, production of the fruiting body is laborious and requires expertise. This research work attempts the production of a food variety; mycomeat through the solid state fermentation of soymilk waste via the fermentative activities of *Lentinus subnudus* mycelia. Its nutritional attributes were then determined.

2. Materials and Methods

2.1 Culture Collection and Maintenance

A pure mycelial culture of *L. subnudus* was obtained from the culture bank of Microbiology unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. It was maintained on PDA slant at 4^{o}C and then sub-cultured at regular time intervals.

2.2 Substrate Treatment

Soymilk waste was prepared according to the method of Akintunde and Akintunde (2002) with a little modification. The moisture content of the soymilk waste was maintained at 60 % and apportioned into four sets. Set L_1 contains soymilk waste alone. Set L_2, in addition to soymilk waste, contains 5 g/l of sucrose, L_3 contains 5 g/l each of sucrose and citric acid, L_4 contains 5 g/l each of sucrose, citric acid and yeast extract. The additives were all constituted externally and were then filled into wide-mouthed transparent jars in triplicates, corked with cotton wool and sterilized in the Autoclave at 121^{0}C for 15 minutes.

2.3 Experimental Set-up

The sterilized substrates were inoculated with the mycelia of *Lentinus subnudus,* a slant was washed per jar. The set-up was then incubated in the dark and monitored daily until full ramification was obtained. The set-up that produced full ramification within the shortest time frame (best mycomeat) was then selected, dried in the oven at 60^{0}C and powdered.

2.4 *Proximate analysis*

The methods of the Association of Official Analytical Chemists (AOAC 2000) were used for the determination of moisture, crude fibre, protein, ash and fat content of the sample. The mycomeat sample was grounded to fine powder and all determinations were done in duplicates. The proximate values were reported in percentage. Mycomeat sample (0.5 grams) in duplicate was used for determination of moisture content by weighing in crucible and drying in oven at 105 ^{o}C, until a constant weight was obtained. Determination of ash content was done by ashing at 550^{o}C for about 3h. The Kjeldah method (AOAC, 2000) was used to determine the protein content by multiplication of the nitrogen value with a conversion factor (6.25). The crude fibre content of the samples was determined by digestion method and the lipid was done by Soxhlet extraction method (AOAC, 2000). Total soluble carbohydrate was determined by the difference of the sum of all the proximate composition from 100%.

2.5 Mineral analysis

The mineral contents of *mycomeat*: calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), Molybdenum (Mo), Vitamin C and copper (Cu) were determined using the atomic absorption spectrophotometer (AAS-Buck 205), as described by the methods of the Association of Official Analytical Chemists (AOAC, 2000). Phosphorus was determined colorimetrically (AOAC, 2000). All the determinations were done in duplicates. The values of calcium, magnesium and potassium were reported in percentages while sodium, iron, zinc, phosphorus, manganese and copper were reported in parts per million (ppm).

2.6 Vitamin C Determination

Vitamin C was determined by iodine titration (AOAC 2000). To 25 mL of mycomeat in a 150 mL beaker was added 35 mL starch-sulphuric acid solution. The resulting solution was titrated with standardised 0.1 M iodine solution (covered from light), while stirring until the first stable blue colour appeared. For the blank, mycomeat was replaced with distilled water. Ascorbic acid (mg/100 mL) was calculated from the formula:
where: Net mL titrant = mL titrant for sample – mL titrant for blank

3.0 Result and Discussion

Soymilk waste supported the growth of the fermenting organism, *L. subnudus* using solid state fermentation technique, with the resultant production of mycomeat. The best mycomeat (Plate 1) was observed with the *L. subnudus* group cultured on soymilk waste with no additive taking only 11 days for full ramification to occur (Table 1), and has a meaty-like aroma than those of its counterpart.. This report is similar to that obtained by Lau et al., (2013), where a growing period of 15 days was observed for the mycelia production of *Lignosus rhinocerotis* through submerged fermentation, as compared to that obtainable for fruit body cultivation, which often takes month(s) to occur. After drying the mycomeat in the oven and powdered, it has a coarse texture with a pH of 3.91 at room temperature.

Proximate analysis revealed in percentages the crude protein content as 14.44±0.01, crude fiber 9.56±0.02, ether extract 15.82±000, ash 5.75±0.01, moisture 2.13±0.01 and carbohydrate as 51.8±1.20 (Table 2). Proximate evaluation of four mushrooms by Okwulehie and Ogoke, (2013) were mostly lower than that obtained in this study, with the exemption of carbohydrate that was higher. The media used for culturing L. subnudus probably contributed to this as media used for the cultivation of mushrooms have effect on their nutritional composition. Carbohydrate being a major source of biological energy in man via their oxidation in cells. Protein consumption enables the involvement of the essential and nonessential amino acids as building blocks for protein biosynthesis, for the constant replacement and turnover of body protein in adult and for the growth of children. Fibre in human diet helps to prevent over-absorption of water, the formation of hard stools which can result in constipation and lowers the body cholesterol level, thus reducing the risk of cardiovascular diseases (Rumeza *et al*., 2006).

Soybean waste materials are generated in large quantities during the processing of soymilk and tofu, and are in some places discarded without treatment thereby constituting an environmental pollutant. In Nigeria, although soymilk waste is been used as livestock feed; however, it spoils very easily and the quantity produced is much more than can be consumed. In this research work, soymilk residue was used as the basal substrate in the cultivation of *Lentinus subnudus* for mycomeat formation by solid state fermentation. The resultant mycomeat formed by growing *L. subnudus* on soymilk residue with no additive appears to have more meat-like consistency as compared to other substrate formulation with additive.

The mineral composition in parts per million is as shown in Table 3. Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body (Eruvbetine 2003). The mineral composition evaluated in percentages showed Calcium which is the principal mineral of bones and teeth as 0.62±0.02%. This study shows the pH of mycomeat as 3.91, which is low enough to facilitate the uptake of the available Calcium in mycomeat if consumed; as excess calcium is found to depress cardiac activity and leads to respiratory and cardiac failure. Sodium is the principal cation in extracellular fluids. It regulates plasma volume and acid-base balance involved in the maintenance of osmotic pressure of the body fluids, preserves normal irritability of muscles and cell permeability. Sodium in this study was shown to occur at 15.90±0.30ppm. Magnesium has many diverse physiological functions. It is essential for the integrity of bone and teeth in the intercellular fluids. Magnesium is an active component of several enzyme systems in which thiamin pyrophosphate (TTP) is a cofactor (McDowell 1992), 0.03±0.01% was found for magnesium.

There is a very high incidence of malnutrition, especially of protein and micronutrient deficiency in developing countries. Micronutrient deficiencies are a major public health problem in many developing countries with infants and pregnant especially at risk (Batra and Seth 2002). Iron deficiency is of greatest public health significance, causing lowered immunity to infections, lowered work capacity and pregnancy complications. Thus, the iron in mycomeat (19.22±0.21ppm) could supplement other iron sources.

Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure and contraction of muscles. Potassium is required during glycogenesis and also in the transfer of phosphate from ATP to pyruvic acid. Potassium deficiency affects the collecting tubules of the kidney, resulting in the inability to concentrate urine, mental confusion and muscular weakness (Murray *et al*. 2000). Thus, the potassium found in this work as 0.06±0.01% is important. Manganese plays a role in energy production and in supporting the immune system (Muhammad *et al.* 2011), manganese was found to make up 8.20±0.04 ppm of mycomeat. Zinc is useful for protein synthesis, normal body development and recovery from illness (Muhammad *et al.* 2011), 7.17±0.01 ppm of Zinc as found in this research work is supplementary.

Copper is an essential micronutrient necessary for the haematologic and neurologic systems (Tan *et al*. 2006). In this work, copper constituent of mycomeat was 2.74±0.02 ppm and that of molybdenum was 0.05±0.01 ppm. Molybdenum is a cofactor for enzymes necessary for the metabolism of sulfur-containing amino acid and nitrogen containing compounds present in DNA and RNA. Molybdenum is a component of several metalloenzymes such as nitrate reductase and hydrogenase. Mycomeat is very rich considering all the nutrients it is enriched with, thus it could be harnessed as another protein-rich “meat” for human consumption. Broadly, the mineral compositions for mycomeat obtained in this work is comparable to that obtained for mushroom and its different developmental stages by Lau et al., 2013, Omar et al., 2011, Musieba et al., 2013 as well as Okwulehie and Ogoke, 2013.

Conclusion

This research revealed that *Lentinus subnudus* can be cultured on soymilk waste for the production of mycomeat. This could in a way reduce pollution of the enviroment resulting from the discharge of soymilk waste into the biosphere. The mycomeat produced could help in nutrient supplication if consumed as meat or used as animal feed. Likewise, the potential of soymilk waste as a good substrate for solid state fermentation technology has been established in this work; especially in the cultivation of *L. subnudus.* Thus, this could be harnessed in the production of aromatic compounds and other secondary metabolites. This study has also established that mycelia cultivation represents a viable source of nutraceuticals and could be harnessed for pharmaceutical purpose. This is because mycelia cultivation is relatively simpler, less problematic, faster, easy to manipulate and economical.

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Plate 1: Mycomeat produced by culturing *L. subnudus* on soymilk residue only

TABLE 1: Degree of meatiness of the mycomeat produced and full ramification days

Group	Degree of meatiness	Ramification days
L_1	++++++	11
L_2	++	19
L_3	++	19
L_4	++++	16

*L_1 : Soymilk residue only; L_2: L_1+5g/l of sucrose
L_3: L_2+citric acid; L_4: L_3+yeast extract
"++++++" – highly meaty; "++++" –moderately meaty ;"++" – not meaty

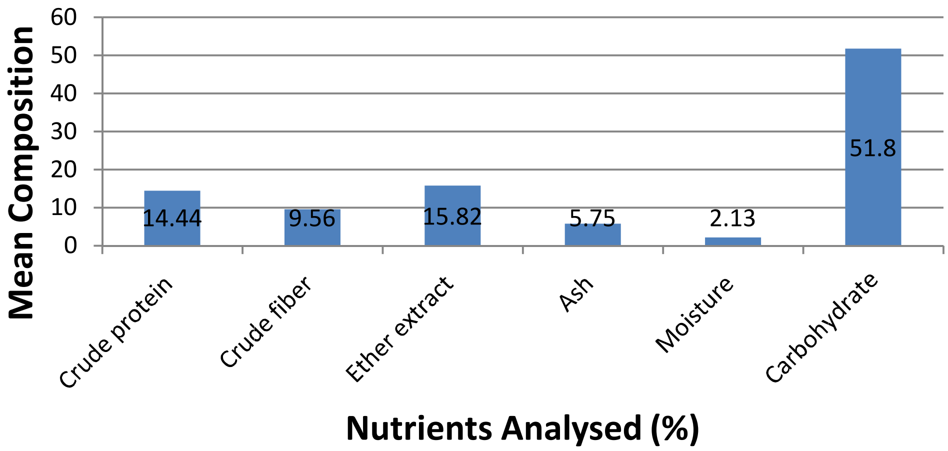

Figure 2: Proximate composition of mycomeat

Table 3: Mineral Composition of mycomeat

Elements (%)	Mean composition ± SD (%)	(ppm)
Calcium	0.62 ± 0.02	-
Magnessium	0.03 ±0.01	-
Potassium	0.06 ± 0.01	-
Vitamin C	0.24 ±0.01	-
Sodium	-	15.90 ±0.30
Manganese	-	8.20 ±0.04
Iron	-	19.22 ±0.21
Copper	-	2.74 ±0.02
Zinc	-	7.17± 0.01
Molybdenum	-	0.05 ±0.01

Legend: *Data are mean values ± standard deviation (SD) of duplicate results;
ppm = parts per million (1mg/kg = 1ppm).