

INFLUENCE OF UV LIGHT EXPOSURE ON MINERAL COMPOSITION
AND BIOMASS PRODUCTION OF *MYCOMEAT* PRODUCED
FROM DIFFERENT AGRICULTURAL SUBSTRATES

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Abstract: The wild and mutant strain of *Pleurotus sajor caju* was cultured on different agricultural substrates. Treatment 1 contained agricultural substrates alone. Treatment 2 contained the mutant strain of the mushroom plus agricultural waste. Treatment 3 contained the wild strain of the mushroom plus agricultural waste. The mutant strain of *Pleurotus sajor caju* cultured on groundnut shell had the highest iron content while the mutant strain cultured on palm kernel meal had the highest biomass production, 10.5 g/L and 17.20 g/L after 7 and 14 days respectively. The proximate analyses of the feed variety (*mycomeat*) revealed the rich nutritional content which may be explored for feed ingredients in livestock production. The findings do not only support the bioremediation of agricultural waste to produce high-value bio-products, but also provide evidence that improvement of microorganism strains represents a viable way to enhance the nutritional value of fermented products.

Key words: agricultural wastes, mutant, *mycomeat*, *Pleurotus*.

Introduction

Agricultural wastes are produced in large quantities during the processing of agricultural products or after harvest and their disposal in developing countries is currently a major economic and ecological challenge. They are usually allowed to accumulate in large quantities, allowed to decay or are burnt indiscriminately which could impact negatively on the environment as well as pose a serious threat to human health. Mushrooms refer to fruiting bodies of macrofungi (Das, 2000)

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and they are highly nutritious (Kumari et al., 2011; Aina et al., 2012). Chang and Miles (1989) coined *mycomeat* to refer to fungal protein obtained through the conversion of food processing biomass wastes; most times via solid state fermentation (SSF). Edible fungi, mainly mushrooms, can be cultured for their fruiting body, metabolites such as an enzyme, or *mycomeat* (containing both the growth substrate and the mycelia of the fermenting fungi). Chiu et al. (2000) have earlier noted that production of medicinal or edible mushrooms is a successful way of agro-waste recycling. Mushroom has been found to suppress breast cancer (Grube et al., 2001). Ultra violet radiation is one of the best physical methods of strain improvement for better performance (Oloke et al., 2012). The microbiological quality of feed is of great importance to its safety. Adetunji and Adejumo (2017) earlier studied the proximate and amino acid profile of *mycomeat* produced from different agricultural substrates. The present study evaluated the nutritional value of a feed variety, *mycomeat*, produced through solid state fermentation from various agricultural wastes using wild and mutant strains of *P. sajor caju*.

Materials and Methods

Pleurotus sajor caju LMU 01 was procured from NIHORT, Ibadan, Nigeria. The cultures were sub-cultured periodically after every 4 weeks and incubated at $25 \pm 1^\circ\text{C}$ for 10 days on potato dextrose agar (PDA) slants and stored at 4°C . A fresh PDA plate to grow the organisms was prepared. After the growth of the organisms, a cork borer was used to obtain several mycelia plugs from the culture into a sterile PDA plate. The sterile plate containing several mycelia plugs was placed under UV lamp at 300 nm wavelength at a distance of 30 cm to the plates. At time interval of 30 min, 5 mycelia plugs were withdrawn and used as inoculants for solid state fermentation studies. The mycelia plugs from the domesticated type culture served as the control (Adetunji and Oloke, 2013).

The basal medium for seed culture was made (w/v) of glucose 2.0%, malt extract 2.0% and peptone 0.1%. The pH was initially adjusted to 5 before sterilizing by autoclaving. *P. sajor caju* was transferred to the medium by punching out 0.7 mm diameter agar discs from a culture grown on PDA plates, and five discs were used to inoculate 100 mL of liquid media. The seed culture was grown in a 250 mL Erlenmeyer flask at 25°C on a rotary shaker incubator at 100 rpm for 3 days. Agricultural wastes (wheat bran [WB], groundnut shell, *Moringa oleifera* seed shell, corn bran [CB], corn cob [CC], palm kernel meal [PKM], rice bran [RB] and cassava peel [CP] meal) which served as substrates were prepared according to the method of Akintunde and Akintunde (2002) with a little modification. The moisture content of the different agricultural wastes was maintained at 60% and apportioned into three sets each. Treatment 1 contained agricultural substrate waste

alone. Treatment 2, in addition to agricultural waste, contained the mutant strain of the mushroom exposed to UV for 30 min. Treatment 3, in addition to agricultural waste, contained the wild strain of the mushroom. They were filled into the wide-mouthed transparent jars in triplicates, corked with cotton wool and sterilized in the autoclave at 121°C for 15 min. The sterilized substrates were inoculated with 10% (v/v) seed cultures on different agricultural substrates. The set-up was incubated in the dark and monitored daily until full ramification was obtained.

The mycelia were obtained by boiling the cultures with boiling water for about 0.5 h to separate the mycelia from the fermented substrate, and filtered immediately with eight layers of gauze. The filter residue was washed three times with boiled water. Then the yield of biomass in SSF was determined gravimetrically after drying at 70°C overnight to a constant weight.

The potassium (K) and sodium (Na) contents of the *mycomeat* were determined using a flame spectrophotometer (Jenway PFP7, UK). The phosphorus content was determined by a UV spectrophotometer (Jenway 6305, UK). The magnesium (Mg) and calcium (Ca) contents were determined by the EDTA titration method while the iron (Fe), manganese (Mn) and copper (Cu) contents were determined using an atomic absorption spectrophotometer (AA320N, PEC Medical, USA) according to the procedure of AOAC (2000). The values of the determined minerals were reported in mg/L. The design of the study was a 3x8 factorial arrangement in a completely randomized design. The mean values obtained for mineral composition and biomass production were subjected to an analysis of variance using SPSS (Version 21). Significant means were analyzed using Duncan's multiple range tests at $\alpha = 0.05$.

Results and Discussion

Using the mutant strain, the *mycomeat* produced from groundnut shell (0.14 mg/L) had the highest iron content while rice bran wild (0.00 mg/L) and cassava peel (CP) control (0.00 mg/L) yielded the least value (Table 1). Conversely, the wild strain using groundnut shell as substrate produced the highest value of calcium content (108.24 mg/L), followed by the mutant strain produced from corn cob, CC (98.52 mg/L) while the wild strain produced from the palm kernel meal, PKM (36.49 mg/L), obtained the least value. The mutant strain produced from the wheat bran, WB (244.52 mg/L), obtained the highest value of magnesium while the wild strain produced from the CC (36.09 mg/L) obtained the lowest values.

The manganese content ranged from 0.04 to 0.23 mg/L. Similar values were obtained by the wild strain produced from the WB (0.04 mg/L), groundnut shell control (0.04 mg/L), the mutant strain produced from groundnut shell (0.03 mg/L), the wild strain produced from groundnut shell (0.04 mg/L), the mutant strain produced from *M. oleifera* (0.04 mg/L), the wild strain produced from *M. oleifera*

(0.03 mg/L), the wild strain produced from corn bran, CB (0.03 mg/L), CC control (0.03 mg/L), the mutant strain produced from CC (0.04 mg/L), the wild strain produced from CC (0.03 mg/L), PKM control (0.03 mg/L), CP control (0.04 mg/L) and the mutant strain produced from CP (0.04 mg/L).

Table 1. Interactive effect of agricultural substrates and strain improvement on mineral composition of *mycomeat*.

Substrates	Strain	Fe	Ca	Mg	Mn	P	K	Cu	Na
WB	Control	0.05g	68.18f	216.45c	0.23a	263.84k	3.88g	0.006def	4.36a
	Mutant	0.02l	47.45q	244.52a	0.09ef	265.79h	3.35i	0.004ef	0.00b
	Wild	0.01m	54.73m	220.46b	0.04h	265.60i	4.63e	0.004ef	0.00b
Groundnut shell	Control	0.10d	88.77c	92.19q	0.04h	229.86p	3.59h	0.003g	0.00b
	Mutant	0.14a	47.42q	98.19p	0.03h	237.97o	4.36f	0.003g	0.00b
	Wild	0.04i	108.24a	166.37f	0.04h	141.25t	3.08j	0.02a	0.00b
<i>M. oleifera</i> seed shell	Control	0.02j	52.29o	138.28i	0.07g	240.96n	1.54m	0.002g	0.00b
	Mutant	0.11c	63.25i	122.26l	0.03h	237.98o	3.10j	0.011b	4.38a
	Wild	0.13b	66.89g	142.31h	0.03h	252.41l	2.59k	0.002g	4.38a
CB	Control	0.003op	46.23r	184.39e	0.08f	329.09b	4.37f	0.009bc	0.00b
	Mutant	0.06f	72.97e	142.29h	0.08f	345.10a	4.88d	0.006def	0.00b
	Wild	0.002pq	58.37j	136.27j	0.03h	277.19f	5.13c	0.002g	0.00b
PKM	Control	0.05gh	41.34s	210.42d	0.03h	302.69c	4.87d	0.02a	0.00b
	Mutant	0.01m	55.96l	126.26k	0.16c	256.28k	5.15c	0.02a	0.00b
	Wild	0.02j	36.49u	156.33g	0.09ef	267.53g	4.64e	0.02a	0.00b
RB	Control	0.00q	38.91t	118.24n	0.12d	294.59d	3.85g	0.002g	0.00b
	Mutant	0.01n	52.39n	88.22s	0.20b	292.86e	3.62h	0.016a	4.38a
	Wild	0.00q	48.66p	102.21o	0.17c	245.23m	2.05l	0.007fg	0.00b
CP meal	Control	0.00 ^q	75.41 ^d	70.18 ^u	0.04 ^h	206.68 ^q	6.42 ^a	0.002 ^g	0.00 ^b
	Mutant	0.02 ^{kl}	98.57 ^b	90.22 ^f	0.04 ^h	182.11 ^s	6.43 ^a	0.003 ^g	4.36 ^a
	Wild	0.07 ^c	57.16 ^k	120.24 ^m	0.12 ^d	191.56 ^t	6.16 ^b	0.003 ^g	4.37 ^a
	SEM	0.01	3.91	11.08	0.01	12.81	0.26	0.001	0.41

Means with different superscripts within the same column are significantly different, P = 0.05, SEM = standard error of the mean; CB = corn bran; WB = wheat bran; RB = rice bran; CC = corn cob; PKM = palm kernel meal.

The mutant strain produced from CB (345.10 mg/L) obtained the highest value of phosphorus while the wild strain produced from CC (106.78 mg/L) obtained the least value. Potassium content was low for *M. oleifera* control (1.54 mg/L) and the wild strain produced from rice bran, RB (2.05 mg/L). The copper content was generally low across the treatments. Sodium was only observed in WB control (4.36 mg/L), the mutant strain produced from *M. oleifera* (4.38 mg/L), the

wild strain produced from *M. oleifera* (4.38 mg/L), CC control (4.35 mg/L), the mutant strain produced from RB (4.38), the mutant strain produced from CP (4.36 mg/L) and the wild strain produced from CP (4.37 mg/L). The groundnut shell (0.094) and *M. oleifera* (0.087) obtained significant higher iron values. Significant lower values of calcium content were reported for PKM (44.60), RB (46.66) and WB (56.79) (Figure 1).

WB obtained the highest value of magnesium (227.14), followed by PKM (164.34). CC (53.45) obtained the least value. Manganese content ranged between 0.16 (RB) and 0.03(CC). Phosphorus content was higher for CB (317.13), RB (277.56) and PKM (275.50). Potassium content ranged between 6.34 (CP meal) and 3.17 (CC and RB). The copper content was generally low. PKM obtained the highest mean (0.022), while CP meal (0.003) obtained the least value. Groundnut seed shell meal, CB, and PKM obtained no values for sodium. *M. oleifera* (2.92) and CP meal (2.91) recorded the highest means, followed by RB (1.46), CC (1.45) and WB (1.45). Strain improvement enhanced mineral contents of the *mycomeat* produced except for magnesium and phosphorus.

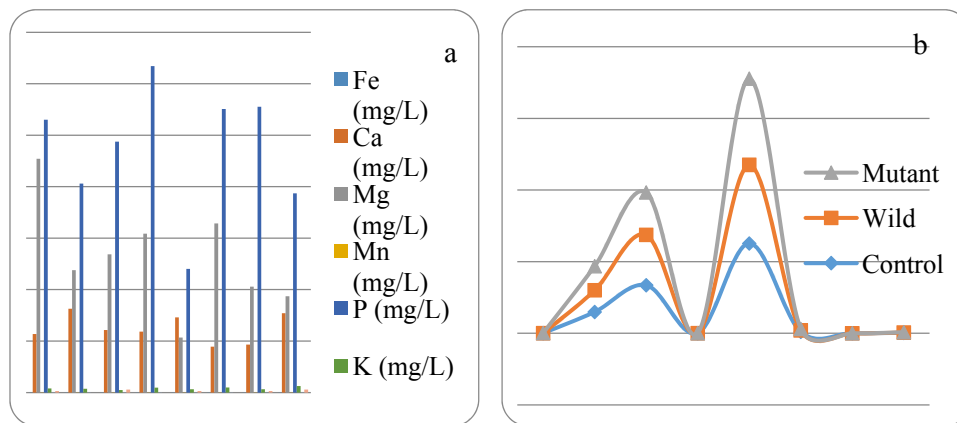


Figure 1. Effects of agricultural substrates and strain type of *Pleurotus sajor caju* on mineral composition of *mycomeat*, A = effect of agricultural substrates on mineral composition of *mycomeat*; B = effect of strain type of *Pleurotus sajor caju* on mineral composition of *mycomeat*.

The mutant strain of the *mycomeat* produced on PKM had the best biomass yield after 7 days (10.50 g/L) and 14 days (17.20 g/L). The main effects of agricultural substrates and strain types on biomass production are presented in Figure 2 while the interactive effect of agricultural substrates and strain improvement on biomass production of *mycomeat* is presented in Table 2. The *mycomeat* produced on palm kernel meal obtained the biomass yield after 7 days

(7.66 g/L) and 14 days (10.03 g/L) while CP obtained the least values. Strain improvement enhanced biomass production.

The low yield by *M. oleifera* seed shell and cassava peel could be due to the inhibitory activities of antibacterial compounds and hydrocyanide acid of *M. oleifera* and cassava peel respectively against the fungi. *M. oleifera* had been previously reported to express anti-microbial properties while cassava is rich in hydrocyanide (Tewe 1994; Ola-Fadunsin and Ademola, 2014).

Table 2. Interactive effect of agricultural substrates and strain improvement on biomass production of *mycomeat*.

Substrates	Strain	7 days (g/L)	14 days (g/L)
WB	Control	0.00 ^j	0.00 ^k
	Mutant	4.20 ^{de}	9.60 ^c
	Wild	2.10 ^g	4.30 ^{fg}
Groundnut shell	Control	0.00 ^j	0.00 ^k
	Mutant	7.20 ^b	13.40 ^b
	Wild	3.20 ^f	6.20 ^e
<i>Moringa oleifera</i> seed shell	Control	0.00 ^j	0.00 ^k
	Mutant	0.03 ^j	0.08 ^k
	Wild	0.00 ^j	0.02 ^k
CB	Control	0.00 ^j	0.00 ^k
	Mutant	1.20 ^{ghi}	4.30 ^{fg}
	Wild	0.90 ^{hij}	2.10 ^{hi}
CC	Control	0.00 ^j	0.00 ^k
	Mutant	3.50 ^{ef}	7.80 ^d
	Wild	1.60 ^{gh}	4.30 ^{fg}
PKM	Control	0.00 ^j	0.00 ^k
	Mutant	10.50 ^a	17.20 ^a
	Wild	8.30 ^b	10.40 ^c
RB	Control	0.00 ^j	0.00 ^k
	Mutant	6.20 ^c	10.40 ^c
	Wild	4.60 ^d	7.80 ^d
CP meal	Control	0.00 ^j	0.00 ^k
	Mutant	0.02 ^j	0.05 ^{jk}
	Wild	0.00 ^j	0.01 ^k
	SEM	0.06	0.09

Means with different superscripts within the same column are significantly different, P = 0.05, SEM = standard error of the mean; WB = wheat bran; CB = corn bran; CC = corn cob; PKM = palm kernel meal; RB = rice bran; CP = cassava peel.

Strain improvement resulted in better biomass yield in the present study. Strain improvement in the field of microbiology is carried out to improve the microbial productivity, to change unused co-metabolites, to improve the use of

carbon and nitrogen sources and to improve the morphology of cells in order to separate the cells and its products.

The values reported in this study for Fe, Ca, Mg, Mn, Na, and Cu are lower than the values reported by Bamigbose et al. (2013). The variation could be attributed to the substrates used. The substrate used in the previous study was richer in these minerals than the substrates used in the present study. Minerals perform structural, physiological, catalytic and regulatory functions in animals. Calcium, phosphorus, and magnesium form structural components of the body organs and tissues. Sodium, potassium, calcium and magnesium in the blood, cerebrospinal fluid, and gastric juice are known to provide maintenance of osmotic pressure, acid-base balance, transmission of nerve impulses and membrane permeability (Suttle, 2010).

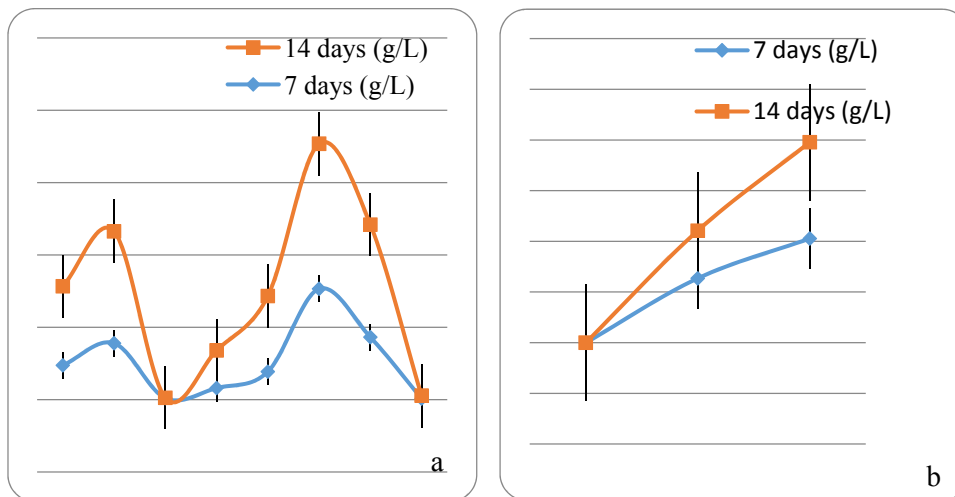


Figure 2. Effects of agricultural substrates and strain type of *Pleurotus sajor caju* on biomass yield of *mycomeat*; A = effect of agricultural substrates on biomass yield of *mycomeat*; B = effect of strain type of *Pleurotus sajor caju* on biomass yield of *mycomeat*.

The values of Mg in the present study are higher than the values reported in the MAFF (1990). The increase in the present study could be attributed to the fermentation and fungal growth. Lack of magnesium in the diet could result in hyperirritability and convulsions. Magnesium is largely protein-bound and functions as a catalyst of a wide array of enzymes. The formation of bone and its maintenance are the most important functions of phosphorus. It is a component of deoxy and ribonucleic acids, essential for cell growth and differentiation (Suttle, 2010).

Conclusion

Strain improvement enhanced iron, calcium, manganese, potassium, copper, and sodium content of *mycomeat*. PKM, groundnut seed shell meal and RB enhanced biomass production of *mycomeat* while *M. oleifera* seed shell meal and CP meal did not. This study supports the bioremediation of agricultural wastes to produce high-value bio-products. It provides evidence that improvement of microorganism strains represents an important means of enhancing nutritional values of fermented products.

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UTICAJ ULTRALJUBIČASTOG ZRAČENJA NA MINERALNI SASTAV I
PROIZVODNJU BIOMASE *MIKOMESA* DOBIJENOG OD RAZLIČITIH
POLJOPRIVREDNIH PODLOGA

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R e z i m e

Divlji i mutirani soj *Pleurotus sajor caju* gajen je na različitim poljoprivrednim podlogama. Tretman 1 se sastojao samo od poljoprivrednog otpada. Tretman 2 je sadržao mutirani soj pečurke i poljoprivredni otpad. Tretman 3 je sadržao divlji soj pečurke i poljoprivredni otpad. Mutirani soj *Pleurotus sajor caju* koji je uzgajan na ljuskama kikirikija imao je najviši sadržaj gvožđa, dok je mutirani soj uzgajan na sačmi od palminog jezgra imao najveću proizvodnju biomase, 10,5 g/L odnosno 17,20 g/L posle 7 odnosno 14 dana. Standardne analize različitih varijanata mikomesa (engl. *mycomeat*) otkrile su bogati hranljivi sadržaj koji bi se mogao koristiti kao sastojak hraniva u stočarskoj proizvodnji. Dobijeni rezultati ukazuju na to da je, pored bioremedijacije poljoprivrednog otpada, moguće dobiti visoko vredne bioproizvode, a takođe pružaju dokaze da poboljšanje sojeva mikroorganizama predstavlja održiv način da se poveća hranljiva vrednost fermentisanih proizvoda.

Ključne reči: poljoprivredni otpad, mutant, *Pleurotus*.

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