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Chemical compositions of *Zea mays* cobs biodegraded by *Lentinus subnudus* and *Pleurotus tuber-regium*

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ABSTRACT

Agro-industrial wastes constitute great nuisance to our environment. These wastes could be recycled into other useful products such as animal feeds and organic fertilizers. Two white rot fungi (*Lentinus subnudus* and *Pleurotus tuber- regim)* were collected from the Botanical Nursery section, University of Ibadan, Nigeria and tissue cultured to generate hyphal starter cultures. Inoculation of active mycelia of these microoranisms into agricultural wastes(maize cobs, *Zea mays*) in a solid state fermentation for 56 days resulted in significant increase (P>0.05) in the crude protein (CP), ether extract (EE) and ash contents (AC) of the fermented wastes. Crude protein (CP)of the maize cob increased significantly from 6.71 g/100g DM control (UM) after 8 weeks to 10.64 and 9.50 g/100g DM for LSM and PT respectively. Likewise, the EE value of UM was 0.37 g/100g DM while that of PT and LSM increased to 1.38 and 0.65 g/100g DM respectively. The dry matter (DM) and crude fibre fractions (CRF) of the treated wastes also reduced significantly in comparism with that of the control (P>0.05). However, acid detergent fractions (ADF) reduced from 47.03 g/100g DM (control) to 41.98 and 40.76 g/100g DM respectively in LSM and PT. In vitro gas production ranged from 0.16ml h^{-1} (UM) to 0.20ml h^{-1} for LSM and 0.11ml h^{-1} for PT. The volume of final gas (a+b) produced also differ in values considerably. For LSM, it was 15.00ml h^{-1}, and 19.67ml h^{-1} for PT but the control (UM) had the value of 11.67ml h^{-1}. Which was significantly lower than the treated cobs(P>0.05). Significant incresed values were also obtained in the fungal treated maize cobs as compared with the control for short chain fatty acid (SCFA), organic matter digestibility (OM) and metabolisable energy (ME). Treatment of maize cobs with the two fungi were also observed to enhance invitrol digestibility in ruminants.

KEYWORDS

Edible fungi;
Biodegradation;
Digestibility;
Maize cob.

INTRODUCTION

Lignocellulosic materials are obtained in large quantities from agricultural and industrial wastes in Nigeria. The poor management of this solid wastes has led to the increasing demand for their possible use in livestock

and animal feeds. Lignocellulose are materials containing high fibre content, low protein, vitamins and minerals[19].

Because Nigeria is a maize producing country, maize cob, straw, husks etc and other wastes from maize are cheaply available after harvest, at the end of each planting season. Chen et al[6] reported that there is a considerable interest in the potential of biologically based process for utilization of such materials for upgrading of animal feeds, in the pulp and paper industry and for the production of chemicals. Livestock in the tropics are being faced with the challenges of poor nutrition. This is because the available crop residues or by- products are of low nutrient quality[2]. Khan et al.[21] and Gbolagade et al.[11,18] independently reported that edible higher fungi are good sources of protein, vitamins and minerals. Mushrooms also contain appreciable amount of potasium, phosphorus, copper, and iron but low level of calcium[8,24]. Mushroom protein intermediates between that of meat and vegetables[7,17].

This good qualities of edible fungi could be incorporated into agricultural wastes for the benefit of livestock. Major agricultural wastes are cereal cobs, straw, leaves, stems, roots etc.[29]. Maize cob is is the remains or left over after the removal of strachy grains. The cob is usually discarded or disposed of and they could cause environmental nuisance when burnt or inadequately dumped. These waste materials could be recycled into value added livestock or ruminant feeds or used as organic fertilizers and the environment is less endangered. Solid state fementation of maize cobs using *Pleurotus tuber regium* and *Lentinus subnudus* will be a cheap recycling technology that can be used by agriculturists in Nigeria.

MATERIALS AND METHODS

Source of agricultural wastes

Seven hunded and fifty kilogrammes (750 kg) of maize cobs were collected from the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. They were sundried for 21days. The materials were pulverised and oven-dried at 55°C to obtain constant weight.

Test fungi

The fruitbodies of *Pleurotus tuber regium* (PT) and *Lentinus subnudus* (LSM) were obtained from decaying log of wood at the back of the Nursery section, Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria. They were subjected to tissue culture to generate mycelial cultures. These were maintained on plates of potato dextrose agar supplemented with 5.0% yeast extract[16].

Substrate preparation and inoculation

One hundred grammes (100.0)g of the dried pulverised substrates were weighed into each 1 Litre heat resistant bottle and 200ml of distilled water were added. The mouth of each bottle was covered with aluminum foil and sterilised in the autoclave at temperature of 121°C and pressure of 1.02 kg cm^{-2} for 20 minutes. Each experiment was replicated thrice.

Seven milimeters (7.0 mm) mycelial disc were removed with the aid of sterile cork borrer from vigorously growing (5day old) cultures of the test fungi. These were used to inoculate each experimental bottle at the center of the substrate and the mouth sealed with aluminium foil. Incubation was done in the laboratory at 28±2ºC and RH of 100%. After 56 days of inoculation, the substrates were harvested[20]. Fungal treated maize cobs were oven dried at 55°C. Chemical analysis and in vitro digestibility were then carried out on the biodegraded samples.

In vitro digestibility

Three West African Dwarf female goat were used. Suction tubes were used to collect intestinal fluid from these goats before they were fed in the morning. Fourteen days prior to the collection of the rumen fluid, these animals have been fed with special diet which was made up of 40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% brewers grain, 1% common salt, 3.75% oyster shell, 0.25% fish meal and 60% guinea grass. This special feeding is necessary so that malnurition will not have negative effect on the composition of rumen fluid collected[9]. Thitrty milimetres (30ml) of strained rumen liquor was added to 200mg of biodegraded sample inside the tightly rapped cheese cloth in a test tube. Buffer was also added to the rumen fluid. The composition of this buffer were: 9.8g $NaHCO_3$, 2.77g Na_2HPO_4, 0.57g KCL, 0.47g NaCL, 0.12g $MgSO_4$. $7H_20$, 0.16g

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$CaCI_{2.}$ $2H_20$. These were added in a ratio (1:4 v/v) under continous flushing with CO_2. (Menke and Steingass,1988). Incubation was carried out at 37°±2°C. The experiment was replicated thee times. The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24h using callibrated syriges[5]. The amount of methane produced was quantitavey estimated by the introduction of 4ml of NaOH (10M) to the already set up experiment. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The volume of gas production characteristics were estimated using the equation $Y = a + b (1 – e^{ct})$ described by Qrskov and McDonald[25], where Y = volume of gas produced at time 't', a = intercept (gas produced from the soluble fraction), b = gas production from the insoluble fraction, a+b= final gas produced, c = gas production rate constant for the insoluble fraction (b), t = incubation time. Metabolizable energy (ME, MJ/Kg DM) and organic matter digestibility (OMD %) were estimated according to the methods described by Getachew et al.[14] and short chain fatty acids (SCFA) was calculated as reported by Getachew et al.[13].

ME = 2.20 + 0.136* GV + 0.057* CP + 0.0029*C;

OMD = 14.88 + 0.88GV + 0.45CP 0.651XA;

SCFA = 0.0239*GV – 0.0601;

GV, CP, CF and XA are net gas production (ml/200mg, DM) crude protein, crude fiber and ash of the incubated sample respectively.

Biodegraded samples nalyses

Crude protein (CP), ether extracts (EE) (crude fat) and ash content(AC) were determined using the procedures of AOAC[1]. Digestible matter (DM) ware determined using the method described by Melaku et al.[23]. The method of Zadrazil[28] was used to assay for hemicellulose. Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were analysed using the method of Van Soest et al.[27] while Acid detergent ligrain (ADL) were quantified using the procudures described by Blummel and Becker[5].

Statistical analysis

All the data derived from the experiments were subjected to analysis of variance (ANOVA) and mean separation were carried out by Duncan's multiple range test using Statistical Analysis System (SAS) package (1988).

RESULTS

TABLE 1 represents results of proximate compositions after 56 days of solid state fermentation by white rot fungi. Crude protein contents of the maize cobs increased significantly (P>0.05) from 6.71 g/100g DM (control) (UM) to 10.64 and 9.50 g/100g DM for *L. subnudus* (LSM) and *P.tuber-regium* respectively (PT) respectively. When compared with the untreated substrates (32.68 g/100g DM), the crude fibre (CF) decreased notably to 26.70 g/100g DM in PT and 17.68 g/100g DM in LSM. Similarly, the dry matter (DM) content of the fermented samples reduced significantly compared with the undegraded cobs. In LSM, 86.66 g/100g DM was observed while in PT, 86.90 g/100g DM was obtained. These values decreased significantly from that of the untreated samples (88.57 g/100g DM). (P<0.05).

There were noticeable increased in the values obtained for the ether extract (EE) and ash content (AC), from untreated substrate (UM) to fungal biodegraded substrates. The value of 0.37 g/100g DM was observed for the UM in EE, whereas 0.65 and 1.38 g/100g DM were recorded for PT and LSM respectively. Similarly, in ash content, UM was 2.86 g/100g DM while 3.16 and 2.98 g/100g DM were observed for LSM and PT respectively. Metabolising energy (ME) also reduced significantly from untreated

TABLE 1 : Nutritional compositions of Fungal biodegraded maize cob((g/100g DM)

Parameters	UM	LSM	PT	SEM
Dry matter	88.57^a	86.66^c	86.90^b	±0.01
Crude protein	6.71^c	10.64^a	9.50^b	±0.04
Ether extract	0.37^c	1.38^a	0.65^b	±0.02
Ash	2.86^b	3.16^a	2.98^b	±0.04
Crude fiber	32.68^a	17.68^c	26.70^b	±0.02
Nitrogen free extract	57.36^b	67.13^a	60.17^b	±0.05
ME (MJ/kg DM)	4958.33^a	4223.60^b	3901.04^b	±1.40

Values followed by different superscripts along each column are significantly varied (P > 0.05) by Duncan's multiple range test. LSM = *Lentinus subnudus* degraded maize cob, UM = untreated maize cob (control), PT = *Pleurotus tuber-regium* degraded maize cob,ME = metabolisable energy

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TABLE 2 : Fiber fractions (g/100g DM) of *Lentinus subnudus and Pleurotus tuber-regium* degraded maize cob

Parameters	UM	LSM	PT	SEM
Neutral detergent fiber	68.35[a]	60.85[c]	61.09[b]	±0.03
Acid detergent fibre	47.03[a]	41.98[b]	40.76[c]	±0.01
Acid detergent ligrain	13.79[a]	11.50[c]	11.77[b]	±0.01
Hemiellulose	33.24[a]	30.48[b]	28.98[c]	±0.00
Nitrogen free extract	57.36[c]	67.13[a]	60.17[b]	±0.05
ME (MJ/kg DM)	4958.33[a]	4223.60[b]	3901.04[b]	±1.40

Values followed by different superscripts along each column are significantly varied (P > 0.05) by Duncan's multiple range test. LSM = *Lentinus subnudus* degraded maize cob, UM = untreated maize cob (control), PT = *Pleurotus tuber-regium* degraded maize cob,ME = metabolisable energy

TABLE 3 : In vitro gas production(ml 200mg DM**) characteristics of biodegraded maize cob**

Parameters	UM	LSM	PT	SEM
a + b (ml)	11.67[c]	15.00[b]	19.67[a]	±1.07
b (ml)	8.00[c]	13.00[a]	12.83[b]	±1.03
y (ml)	9.67b	11.67[a]	9.00[b]	±1.07
c (h^{-1})	0.16[b]	0.20[a]	0.11[c]	±0.01

Values followed by different superscripts along each column are significantly varied (P > 0.05) by Duncan's multiple range test. LSM = *Lentinus subnudus* degraded maize cob, UM = untreated maize cob (control), PT = *Pleurotus tuber-regium* degraded maize cob,ME = metabolisable energy

TABLE 4 : Short chain fatty acid (mol organic matter digestibility (%),) and metabolisable energy (MJ/Kg DM) of treated maize Cob

Parameters	UM	LSM	PT	SEM
SCFA	0.34[b]	0.42[a]	0.41[a]	±0.10
OMD	28.44[b]	33.14[a]	28.87[b]	±1.08
ME	4.26[b]	4.88[a]	4.27[b]	±1.10

Values followed by different superscripts along each column are significantly varied (P > 0.05) by Duncan's multiple range test. LSM = *Lentinus subnudus* degraded maize cob, UM = untreated maize cob (control), PT = *Pleurotus tuber-regium* degraded maize cob,ME = metabolisable energy. OMD=Organic matter digestibility; SCFA=Short chain fatty acid;ME=metabolisable energy

substrate (4958.33 MJ/KgDM) to 4223.60 and 3901 MJ/Kg DM in LSM and PT respectively.

Fibre analysis of the biodegraded samples was presented on TABLE 2. Apart from the nitrogen free extract (NFE), there were general decrease in the fibre fractions of maize cob. The values for acid detergent fibre (ADF) reduced from 47.03 g/100g DM in the control experiment (UM) to 41.98 and 40.76 g/100g DM in LSM and PT g/100g DM.The same trend of significant reduction were observed in the NDF, ADL

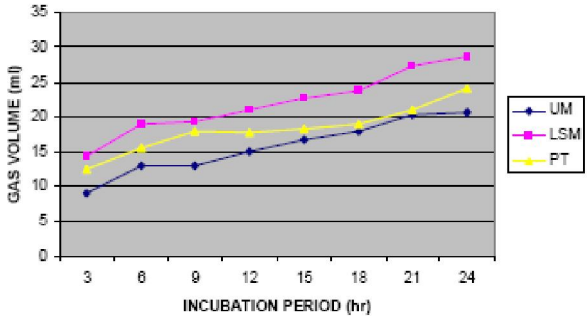

Figure 1 : In vitro gas production pattern of UM, PSM and PT over a period of 24 hours

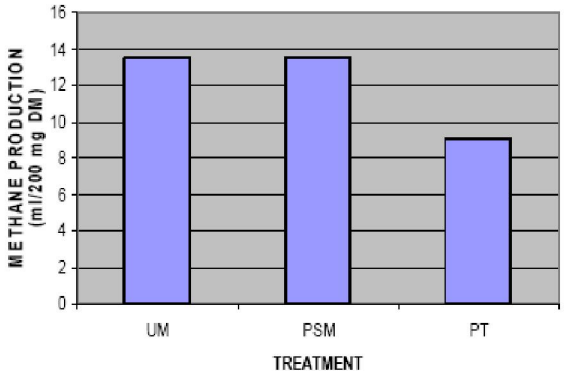

Figure 2 : Methane production from in vitro gas production of maize cob treated with two strains of mushroom UM=control PSM=*Lentinus subnudus;* PT=*Pleurotus tuber-regium*

and cellulose contents of fungi treated maize cob. (P>0.05). Hemicellulose values also reduced significantly and showed marked statistical (P>0.05) different in the control (UM), *L. subnudus* and *P.tuber-regium*. It was observed that nitrogen free extract (NFE) was 57.36 g/100g DM in the control experiment. This values increased significantly (P<0.05) in PT (60.17 g/100g DM) and LSM(67.13 g/100g DM) after the biodegradation of maize cob.

TABLE 3 shows the in vitro gas production characteristics of maize cob over a period of 24 hours. Gas production (b) (ml 200mg DM) apparently varied between samples from 80.00ml (control) UM to 13.00ml (LSM) and 12.83 (PT). The gas production rate (c) (mlh^{-1}) also differed significantly (p<0.05). The fractional rate ranges from 0.16ml h^{-1} (control) UM to 0.20ml h^{-1} (LSM) and 0.11ml h^{-1}(PT). There were significant difference (P<0.05) in the final gas produced (a+b) ml. The highest value was 19.67ml was obtained in PT.This value reduced significantly in LSM (15.00ml) while the lowest value of 111.67 ml was obtained in the control (UM).

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There were no significant differences (P>0.05) in the values obtained for UM, LSM and PT.for short chain fatty acid, (SCFA) (TABLE 4). There were significant variations (p<0.05) in OMD of the LSM (33.14 MJ/KgDM). fungal treated maize cob compared with the control (28.44 MJ/KgDM)), but the values obtained in control and PT (28.87MJ/KgDM). are not significantly different. The proportion of SCFA was significantly higher in LSM (0.42 MJ/KgDM). and PT (0.41MJ/KgDM), than the control (0.34 MJ/KgDM). with the highest proportion recorded in LSM. The ME of the control and PTwere statistically similar (p>0.05), however, it was significantly different () from LSM. Figure 1 shows the in vitro gas production pattern of the (control) UM, LSM and PT over a period of 24 hours while figure 2 shows the bar chart of methane production in the substrates treated by these two fungi.

DISCUSSION

The progressive reduction observed in crude fibre, and crude fibre fractions in the fungal treated wastes may be attributed to the biodegradation of cellulose and hemicellulose by these two white rot fungi[6]. The breakdown of these complex compounds by *Pleurotus tuber-regium* and *Lentinus subnudus* may be linked with thier ability to produce lignocellulosic enzymes which are capable of bioconversion of lignin, cellulose and hemicellulose to other simple digestable materials[15,20].

The progresive increament in the crude protein (CP) content noticed in fungal fermented substrates may be attributed to the addition of fungal biomas to the fermented samples[6]. The established fungal hyphae observed in the biodegraded maize cobs may also be linked with the improved crude protein values obtained in the fermented agricultural wastes[21]. Mushroom vegetatative hyphae have been suggested to be good sources of proteins and essential mineral nutrients[8,24]. The noticable higher values of CP obseved in the maize cobs after solid state fermentation for 56 days could also be due to the release of the polysaccharide bound proteins and this may enhance the fermented substrates to have more nutritional quality[4]. The progressive reduction recorded in the Neutral detergent fibre (NDF) within the 56 days of biodegradation may be due to the extensive utilization of hemicellulose by *P.tuber-regium* and *Lentinus subnudus*. This observation is in support of earlier work of Chen et al.[6], on biodegradation of agricultural wastes using other fungi. The crude fibre (CF) and acid detergent fibres (ADF)values which reduced gradually within the 8 weeks of biodegradation in the treated substrates could be favourably compared with the earlier work of Jonathan *et al*.[20] who also recorded similar reduction in CF and ADF in the fungal treated agro-industrial wastes.

The reduction observed in cellulose content in the biodegraded cobs could be linked to utilization of cellulose for fungal metabolism[10]. With the exception of gas production, c(h^{-1}), that was high in control experiment (UM), final gas production, (a+b)ml, volume of gas produced, y (ml), and gas production from the insoluble fraction., b (ml) were better in the biodegraded wastes This is an indicator that the cobs undergone very good solid state fermentation. However, final gas produced, (a+b)ml, volume of gas produced, y (ml), and gas production from the insoluble fraction., b (ml) were all better in *Lentinus subnudus*(LSM) than in *Pleurtus tuber-regium*(PT) this may be attributed to the higher cellulose content recorded for latter. It has been suggested that cellulose could serve as an energy souce for vegetative growth of fungi[16]. Also, the higher hemicellulose content recorded for LSM will provide more glucose for the ruminant animals since the gut of the animals are rich with microorganisms that could convert cellulose to glucoseby the help of lignocellulosic enzmes[3]. The different types of white rot fungi used may be responsible for the varied gas production rate constant, c(h^{-1} observed in PT and LSM. The best Short chain fatty acid (SCFA) values were recorded for the biodegraded substrates copared with the untreated (control)t (P>0.05). This result indicated the potentials of making energy freely availabe to the ruminants using maize cobs fungal treated samples[20]. The improvement in the CP coupled with the decrease in the CF and CF fractions of LSM and PT will be the most likely reason that contributed to the improved organic matter digestibility (OMD). Methane (CH_4) production represents energy loss, since the values obseved in LSM and PT were lower than the values observed in control (UM). It could be seen from these investigations that the values CP, EE, AC, SCFA and OMD in the biodegraded maize cobs significantly improved than that

of the control. This actually suggested that the fungal treated maize cobs could serve as the potentially useful products for animal feeds.

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