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In vitro Rumen Fermentation Characteristics of White Rot Fungi Biodegraded Cassava (*Manihot esculenta*) Peels

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was designed to evaluate the chemical composition, *In vitro* gas production, fermentation characteristics, methane produced and estimated metabolisable energy, organic matter digestibility and short chain fatty acids of untreated cassava peels and white rot fungi (*Pleurotus osetreatus, Pleurotus eryngii, pleurotus tuber- regium* and *Lentinus edodes*) biodegraded cassava peels as potential feedstuffs.
Study Design: Experimental design employed was complete randomized design.
Place and Duration of the Study: The study was conducted at the Farm Unit of the College of Agriculture, Lafia, Nigeria and Animal Science laboratoryof University of Benin, Nigreia. The duration of study was 21 days.
Methodology: Crude protein was determined as Kjeldahl nitrogen x 6.25. Ether extracts, crude

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fibre and ash were determined in triplicates. *In vitro* gas production using rumen fluid obtained from West African Dwarf goats and incubation was carried out. The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h. After 24 hours of incubation, methane produced was estimated and determined in triplicates.

Results: The proximate composition of the biodegraded cassava peels samples indicated *Pleurotus tuber-regium,* as most efficient amongst the four white rot fungi in significantly improving the lignocellulosic cassava peels. Superior (P<0.05) proximate values were recorded for *pleurotus tuber- regium* biodegraded cassava peel (PT-CPS) compared to all other fungi biodegraded cassava peel samples. Untreateted cassava peel (UCPS) was improved by this fungus from crude protein of 3.33 to 9.83%; crude fibre content reduced from 18.61 to 10.66%; and better value of ash (from 4.00 to 5.84%). Similarly, significant (P<0.05) values of IVGP (20.00ml/200mgDM), ME (5.21 MJ/kgDM), OMD (40.70%), SCFA (0.4179 µmol) and CH_4 estimate of 10.33ml/200 mgDM were obtained for *pleurotus tuber- regium* biodegraded cassava peel (PT-CPS).

Conclusion: Results obtained in this study inferred that *Pleurotus tuber-regium* as the most efficient fungus in improving the nutritive value of cassava peels.

Keywords: White rot fungi; cassava peels; biodegradation; In vitro rumen fermentation.

1. INTRODUCTION

The enormous cassava peel (*Manihot esculenta*) waste [1] accruing from processing the tubers in Nigeria is generally considered to contribute significantly to environmental pollution and aesthetic nuisance [2]. The wastes generated at present in Nigeria pose a disposal problem (with projected annual production of 60 million tonnes [1] and would even be more problematic in the future with increased industrial production of cassava products.

The use of microorganisms to convert carbohydrates, lignocelluloses and other industrial wastes into foodstuffs rich in protein is possible due to the ability of microorganisms to grow very fast [2], hence they can be easily modified genetically for growth on a particular substrate under particular cultural conditions to produce high protein content varying from 35 to 60% [2]. The ability to grow in slurry or on solids and their nutritional values makes them as good as other conventional foods rich in protein.

In vitro digestibility techniques provide a quick, inexpensive, and precise prediction of *in vivo* or conventionally determined digestibility in ruminants [3]. The *In vitro* procedure does a better job of prediction than chemical composition because it accounts for all factors affecting digestibility, whether known or unknown, which is not possible with current chemical methods. Gas measurement *In vitro* digestion focuses on the appearances of fermentation products (soluble but not fermentable products do not contribute to gas production). In the gas method, kinetics of fermentation can be studied on a single sample and therefore a relatively small amount of sample is required or a larger number of samples can be evaluated at a time [4].

Studies conducted by some workers [5-8] provided information on the potentials of converting agricultural waste into value added feedstuffs for ruminant with some species of white-rot fungi (*Pleurotus ostreatus*, *Pleurotus tuber-regium, Pleurotus pulmonarius, Pleurotus sajorcaju* and *Lentinus subnudus*). These studies showed significant (P<0.05) increase in crude protein content and significant (P<0.05) decrease in cellulose content of the wastes. Their work also showed potential of fungal treatment in improving crude protein, short chain fatty acid value, metabolizable energy and enhanced digestibility potential of the by-products by white rot fungi for the ruminant animal [9].

This study was designed to evaluate the chemical composition, *In vitro* gas production, fermentation characteristics, methane produced and estimated metabolisable energy, organic matter digestibility and short chain fatty acids of untreated cassava peels and white rot fungi (*Pleurotus osetreatus, Pleurotus eryngii, pleurotus tuber- regium and Lentinus edodes*) biodegraded cassava peels as potential feedstuffs.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted at the Farm Unit of the College of Agriculture, Lafia, Nigeria located

Latitude N08°29′8.66″; Longitude E08°29′49.10″ and Altitude 164.5 m; in the guinea savannah vegetation, with its sandy loam soil texture [10].

2.2 Sample Collection and Inoculation

Four sample weighing 100 g sample each of sun dried cassava peels (collected from the Family Support Programme's Gari Processing industry, Shabu, Lafia and sun dried for six days as described by [11] were mixed thoroughly in 250ml conical flasks with 5 ml each of isolated liquid white rot fungi species (*Pleurotus ostreatus*, *Pleurotus tuber-regium, Pleurotus eryngii,* and *Lentinus edodes*) obtained from Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, Nigeria. Each inoculated flask was clogged with cotton and covered with foil; they were kept in the dark cupboard in the laboratory at 30°C and 100% relative humidity (RH). After 21 days of inoculation, the experimental bottles were harvested after autoclaving (at 120°C for 15 minutes) to terminate the mycelia growth. Samples of the untreated and biodegraded cassava peel samples were oven dried to constant weight for chemical analysis and *In vitro* digestibility.

2.3 Chemical Composition

DM was determined by oven drying the milled samples to a constant weight at 105°C for 8 hours. Crude protein was determined as Kjeldahl nitrogen x 6.25. Ether extracts, crude fibre and ash were determined according to [12] method.

2.4 *In vitro* Gas Production and Fermentation Characteristics of Fungi Treated and Untreated Cassava Peels

Rumen fluid was obtained from West African Dwarf goats through a suction tube before offering the morning feed. Incubation was carried out according to [13] in 120 ml calibrated syringes in three batches at 39°C. To 200mg sample in the syringe was added 30ml inoculums containing cheese cloth strained rumen liquor and buffer (9.8 g $NaHCO_3$ + 2.77 g Na_2HPO_4 + 0.57 g KCL + 0.47 g NaCl + 0.12g $MgSO_4$. $7H_20$ + 0.16 g $CaCl_2$. $2H_20$) at a ratio of 1:4 v/v under continuous flushing with CO_2.

The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h. After 24 hours of incubation, 4 ml of NaOH (10 M) was introduced to estimate the amount of methane produced according to the methods described by [14]. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

The gas production characteristics was estimated using the equation $Y = a + b\ (1\text{-}e^{ct})$ described by [15], 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.

The post incubation parameters such as metabolisable energy (ME, MJ/kg), organic matter digestibility (OMD %) and short chain fatty acids (SCFA) was estimated at 24h post gas collection according to [10], 1988: ME = 2.20 + 0.136Gv + 0.057CP + 0.0029CF; OMD = 14.88 + 0.88Gv + 0.45CP+ 0.651XA; SCFA = 0.0239Gv – 0.0601. Where Gv, CP, CF and XA are net gas production (ml/200 mg, DM), Crude protein, Crude fibre and ash of the incubated sample, respectively.

2.5 Dry Matter Degradability Determination

The post incubation samples were poured into the respective test tubes for centrifugation in order to obtain the residues. The residue was recovered after thorough washing with clean water and later oven-dried to a constant weight as described by [14]. The final and initial weights recorded were used to calculate dry matter degradability.

2.6 Statistical Analysis

Experimental design employed was complete randomized design [16]. Data obtained were subjected to analysis of variance when significant differences occurred, the means were separated using Duncans' multiple range test [17].

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Untreated and Fungal Biodegraded Cassava Peels

The four fungal treatments (Table 1) had significantly ($P<0.05$) increased CP content of cassava peels. But among the different fungal treatments, CP content was higher for PT-CPS

(9.83%) and PO-CPS (9.71%) than for LE-CPS and PE-CPS. The ability of these fungi to improve the CP content from untreated CPS 3.33% was significantly (P<0.05) obvious, showing an increased CP contents of 6.50% and 6.38% for PT-CPS and PO-CPS respectively over UCPS attributed to an increase in mycelial biomass. The Variation in the CP content could also be dependent on the plant residues and fungi species [18,8]. Other reasons for the increased CP content of fungi biodegraded cassava peels in this study could be the capture of excess nitrogen of aerobic fermentation by the microorganisms inferred by some previous workers [5,19,20].

Sallam [21] had deduced that the biodegradation of fibrous material releases the polysaccharide bond which is the first step towards increasing the nutritive value of any fibrous material. Fungal treatment significantly (P<0.05) decreased CF content from 18.61% (UCPS) to 10.66 (PT-CPS), 11.067% (LE-CPS), 11.25% (PO-CPS) and 13.41% (PE-CPS). These decreases in CF with biodegradation could be ascribed to hydrolytic nature of the fungi species which could have disrupted the cell wall and improved degradation of CF. Among the edible white-rot fungi, the *Pleurotus* species have been shown to be more efficient in CF degradation [22,23].

The CF portion of the diet material could represents the indigestible fiber fraction and NFE supposed to represent the more readily digestible carbohydrates; in this study, the NFE was significantly (P<0.05) affected (Khan et al., 3003). NFE of untreated CPS is 63.80% while biodegraded samples shows decreased values of NFE but no significant (P>0.05) difference among the biodegraded samples. The variation in chemical analyses for CF and NFE probably justifies the development of detergent fiber system.

Ash content in the biodegraded samples significantly (P<0.05) improved with 1.84%, 1.68%, 1.75% and 1.94% for PT-CPS, LE-CPS, PO-CPS and PE-CPS respectively. The improved ash value could be ascribed to the rich mineral nature of edible fungi used in this study as suggested [24,25] that some of the mineral are already part of the mycelia biomass of the various white rot edible fungi used.

A significant (P<0.05) decrease in the DM of the biodegraded cassava peels was observed from 90.60% to: 87.57% (PT-CPS), 87.74% (LE-CPS), 87.66% (PO-CPS) and 88.74% (PE-CPS).

3.2 Effect of Fungal Biodegradation on Fermentation Characteristics of Cassava Peels

The first gas production (a) was significantly (P<0.05) highest for PO-CPS (7.30ml/200 mgDM) and least for PE-CPS (3.00 ml/200 mgDM), While PT-CPS and LE-CPS had similar values of 6.00 and UCPS gave 4.67ml/200 mgDM. This trend shows gas production from soluble part of the substrates fermented indicating PO-CPS has more soluble fraction as deduced by [25]. Final gas production (a+b) showed no significant difference but PE-CPS obtained in this study indicates the least insoluble fractions and the least biodegraded of the substrates.

Table 1. Effect of fungal biodegradation on proximate composition of cassava peels

%	UCPS	PT-CPS	LE-CPS	PO-CPS	PE-CPS	SEM
CP	3.33^{d}	9.83^{a}	9.22^{b}	9.71^{a}	5.96^{c}	0.69
EE	0.99^{d}	0.91^{d}	1.03^{b}	0.97^{c}	2.92^{a}	0.21
CF	18.61^{a}	10.66^{e}	11.67^{d}	11.25^{c}	13.41^{b}	0.79
NFE	63.80^{a}	60.31^{b}	60.65^{b}	60.35^{b}	60.52^{b}	0.37
Ash	4.00^{e}	5.84^{b}	5.68^{d}	5.75^{c}	5.94^{a}	0.19
DM	90.60^{a}	87.57^{d}	87.74^{c}	87.66^{d}	88.74^{b}	0.31

a,b,c,d,e – Means on the same row with different superscripts are significantly (P<0.05) different
SEM – Standard Error of Mean, CP –Crude Protein, EE –Ether Extract, CF –Crude Fibre, NFE –Nitrogen Free Extract, UT-CPS Untreated Cassava Peels, PE-CPS Pleurotus tuber regium treated Cassava Peels, LE-CPS Lentinus edodes treated Cassava Peels, PO-CPS Pleurotus ostreatus treated Cassava Peels, PE-CPS Pleurotus eryngii treated Cassava Peels

Gas production from insoluble fraction was significantly (P<0.05) highest for PT-CPS (14.00ml/200mgDM). This could mean PT-CPS was the better biodegraded of the four white rot fungi degraded substrates. The factor that determined amount of gas produced during fermentation in this study could be the nature of the fibre, protein richer substrate and the fungi species. The finding in this study agrees with that of [26] that high crude protein in feed material enhances microbial multiplication in the rumen which determines extent of fermentation. Gas production constant for insoluble fraction by PT-CPS indicates a superior (P<0.05) constant of 0.0095 as seen in Table 2.

3.3 Effect of Fungal Biodegraded Cassava Peels on IVGP, ME, OMD, SCFA and Methane Estimates

The IVGP (ml/200mgDM) values obtained for UCPS (18.00), PT-CPS (20.00), LE-CPS (18.00) and PO-CPS (19.00) significantly (P<0.05) differed from 10.00 recorded for PE-CPS (Table 3). The difference could be attributed to the hydrolytic ability of the fungi species, nature of carbohydrate and potency of the rumen liquor used for incubation [26,27]. The effect of degradation by fungi improved nutrients which enhance fermentations and production of more gas (Fig. 1) as gas production is a function and mirror of degradable carbohydrate.

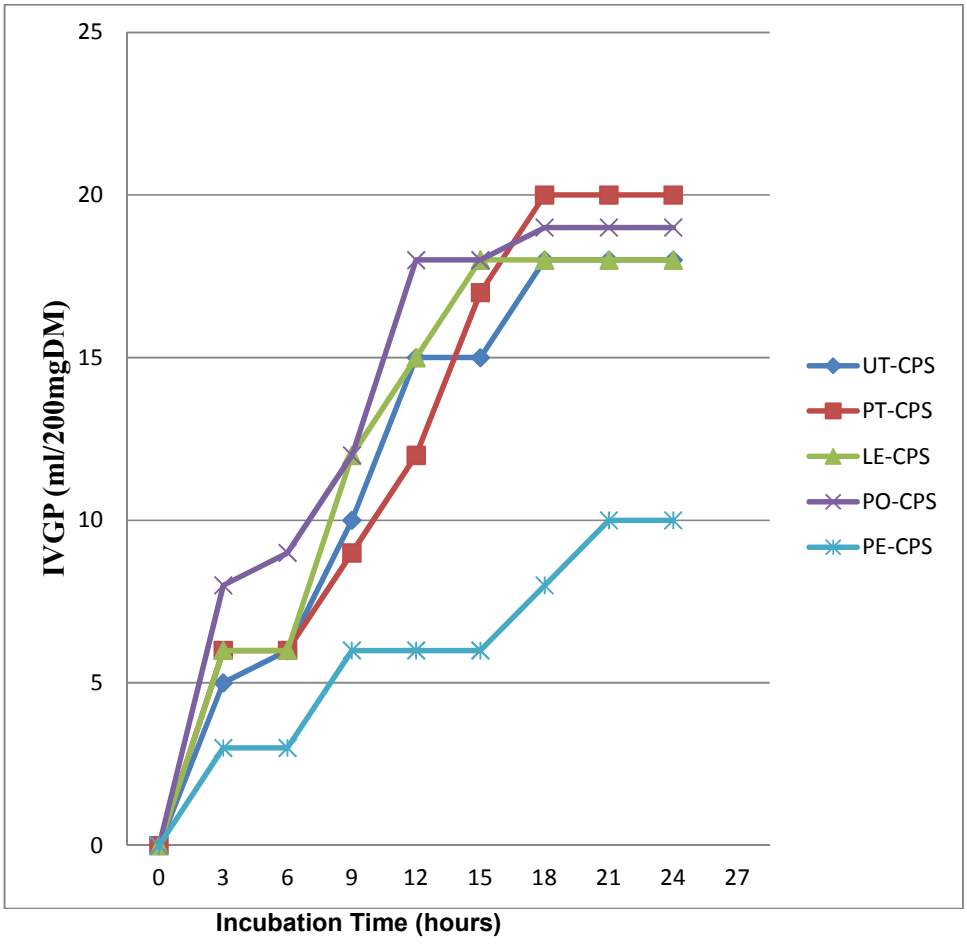

Fig. 1. IVGP of untreated and fungal biodegraded cassava peels

UT-CPS: Untreated Cassava Peels, PE-CPS: Pleurotus tuber regium treated Cassava Peels, LE-CPS: Lentinus edodes treated Cassava Peels, PO-CPS: Pleurotus ostreatus treated Cassava Peels, PE-CPS: Pleurotus eryngii treated Cassava Peels

The similar trend of significance (P<0.05) obtained for values of ME (Mj/kgDM), OMD (%), SCFA (µmol) and methane (ml/200mgDM) shows different potentials of the fungi and the capacity of a high gas production feed material to synonymously produce methane which is an energy waste to the ruminant animal (Fig. 2). The result in this study has been corroborated by some authours [27,28] that feedstuffs that show high capacity for gas production have been implicated to increase methanogenesis.

Fig. 3 shows percentage DM degradability of fungal biodegraded cassava peels and untreated cassava peels; the DMD percentages of 23.93, 84.52, 60.80, 56.50 and 42.08% were obtained for UCPS, PT-CPS, LE-CPS, PO-CPS and PE-CPS respectively. The lignin- degrading ability of these various white rot fungi could be simply put as shown in Fig. 3.

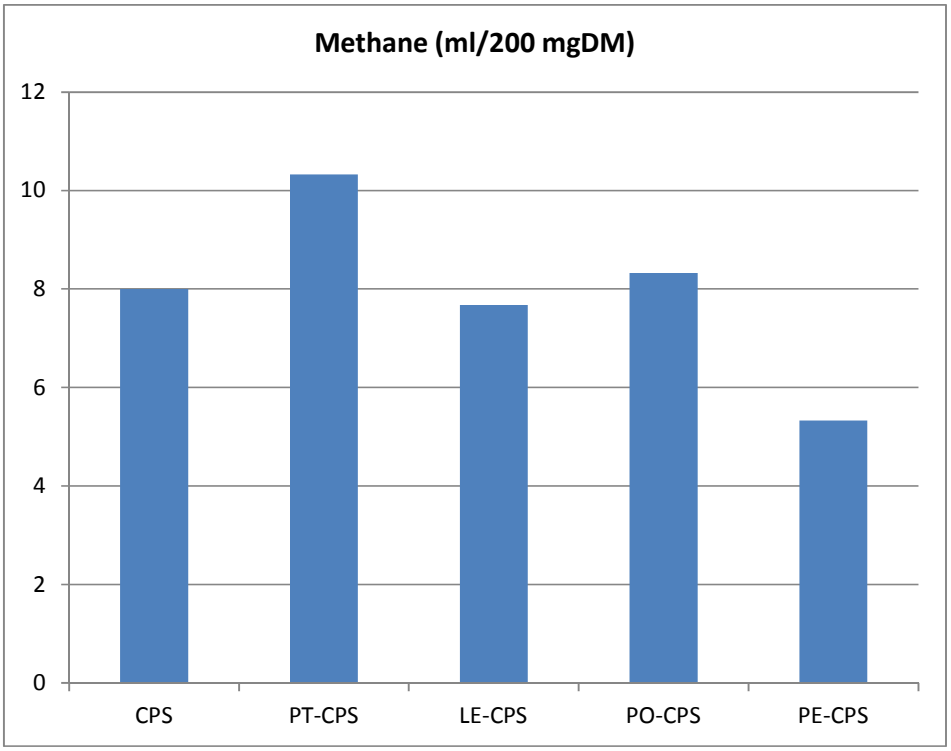

Fig. 2. Methane produced after 24 hours fermentation of untreated and fungal biodegraded Cassava peels

CPS - Untreated Cassava Peels, PE-CPS - Pleurotus tuber regium treated Cassava Peels LE-CPS - Lentinus edodes treated Cassava Peels, PO-CPS - Pleurotus ostreatus treated Cassava Peels, PE-CPS - Pleurotus eryngii treated Cassava Peels

Table 2. Effect of fungal biodegradation on fermentation characteristics of Cassava peels for 24 hours

	UCPS	PT-CPS	LE-CPS	PO-CPS	PE-CPS	SEM
a	4.67^{bc}	6.00^{ab}	6.00^{ab}	7.30^{a}	3.00^{c}	0.45
(a+b)	18.00^{a}	20.00^{a}	18.00^{a}	19.00^{a}	10.00^{b}	0.96
b	13.33^{b}	14.00^{a}	12.00^{bc}	11.33^{c}	7.00^{d}	0.68
c	0.0189^{bc}	0.0095^{c}	0.0556^{ab}	0.0480^{b}	0.0635^{a}	0.01

a,b,c,d,e – Means on the same row with different superscripts are significantly (P<0.05) different a – First Gas Production, (a+b) – Final Gas Production, b – Gas Production from Insoluble Fraction, c – Gas production Constant for Insoluble Fraction(b), SEM – Standard Error of Mean, UT-CPS Untreated Cassava Peels, PE-CPS Pleurotus tuber regium treated Cassava Peels, LE-CPS Lentinus edodes reated Cassava Peels, PO-CPS Pleurotus ostreatus treated Cassava Peels, PE-CPS Pleurotus eryngii treated Cassava Peels

Table 3. Effect of fungal biodegradation of Cassava peels on IVGP, ME, OMD, SCFA and methane estimates

	UCPS	PT-CPS	LE-CPS	PO-CPS	PE-CPS	SEM
IVGP (ml/200 mgDM)	18.00[a]	20.00[a]	18.00[a]	19.00[a]	10.00[b]	0.96
ME (MJ/KgDM)	4.89[d]	5.51[a]	5.21[c]	5.39[b]	3.94[e]	0.15
OMD (%)	34.82[d]	40.70[a]	38.77[c]	39.71[b]	30.08[e]	1.05
SCFA (µmol)	0.3701[b]	0.4179[a]	0.3701[b]	0.3940[b]	0.1789[c]	0.03
Methane (ml/200 mgDM)	8.00[b]	10.33[a]	7.67[b]	8.33[b]	5.33[c]	0.44

a,b,c,d,e – Means on the same row with different superscripts are significantly (P<0.05) different
IVGP – In vitro Gas Production, ME – Metabolisable Energy, OMD – Organic Matter Digestibility, SCFA – Short Chain Fatty Acids, SEM – Standard Error of Mean, UT-CPS – Untreated Cassava Peels, PE-CPS – Pleurotus tuber regium treated Cassava Peels, LE-CPS – Lentinus edodes treated Cassava Peels, PO-CPS – Pleurotus ostreatus treated Cassava Peels, PE-CPS – Pleurotus eryngii treated Cassava Peels

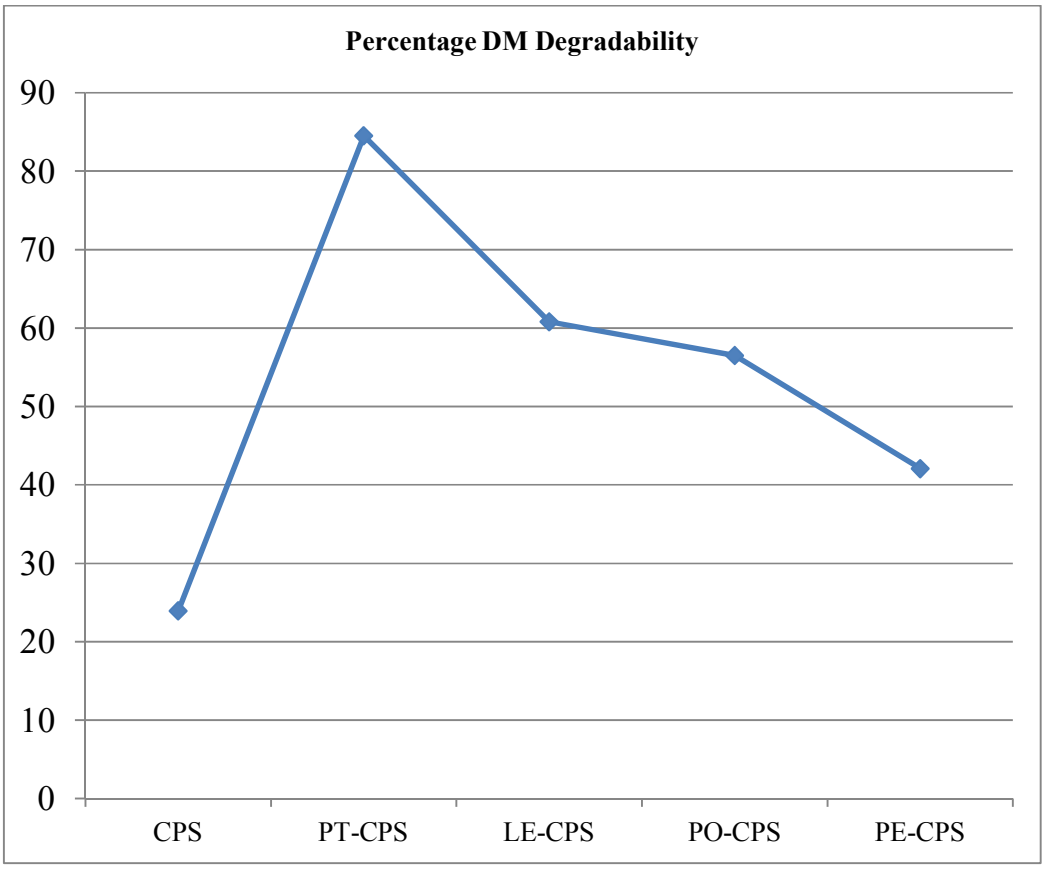

Fig. 3 . Percentage DM degradability of fungal biodegraded cassava peels and cassava peels
CPS - Untreated Cassava Peels, PE-CPS - Pleurotus tuber regium treated Cassava Peels LE-CPS - Lentinus edodes treated Cassava Peels, PO-CPS - Pleurotus ostreatus treated Cassava Peels, PE-CPS - Pleurotus eryngii treated Cassava Peels

4. CONCLUSION

The proximate composition of the biodegraded cassava peels samples indicated *Pleurotus tuber-regium,* as most efficient amongst the four white rot fungi in significantly improving the lignocellulosic cassava peels. Cassava peels was improved by *Pleurotus tuber-regium* fungus from crude protein of 3.33 to 9.83%; crude fibre from 18.61 to 10.66%; and better values of Ash (from 4.00 to 5.84%). Results showed significantly better IVGP (20.00 ml/200 mgDM), ME (5.51 MJ/kgDM), ODM (40%), SCFA (0.4179 µmol) and the wasteful methane of 10.33ml/200

mgDM of *Pleurotus tuber-regium* biodegraded cassava peels. The data obtained in this study inferred *Pleurotus tuber-regium* as the most efficient in improving the nutritive value of cassava peels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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