

**BIODEGRADATION OF CASSAVA PEEL WITH *Pleurotus ostreatus* AND *Bacillus cereus*
FOR THE PRODUCTION OF POULTRY FEED**

ABSTRACT

This study aimed to produce poultry feed from *Pleurotus ostreatus* and *Bacillus cereus*. biodegraded cassava peel. The biodegradation of cassava peel substrates was for 28 day. The biodegradation setups ST1-ST9 consist of 150g cassava peel + 30 ml, 20ml and 10ml of Consortium, *Bacillus cereus* and *Pleurotus ostreatus* respectively. Treated cassava substrate was fortified with palm press fiber (PPF) and fish waste (FW). The birds were fed with the feeds and monitored for 28 days. Results for proximate analysis of the fortification materials showed the nutrient concentration as: Moisture (PPF=7.1%, FW=2.75%); Ash (PPF=1.45%, FW=29.9%); Lipid (PPF=45.24%, FW=7.88%); Carbohydrate (PPF=3.35%, FW=6.33%); Fibre (PPF=28.42%, FW=11.6%) and Protein (PPF=14.44%, FW=41.56%). Improved proximate composition was noticed in poultry feeds ST1, ST4 and ST9 after fortification, with ash values of 8.50, 5.70 and 6.90; carbohydrate 21.12, 28.25 and 27.72; lipid 8.50, 6.70 and 7.90; protein 19.31, 20.25 and 18.13, and fibre 29.77, 30.00 and 28.05 in that order. Results of growth parameters of birds fed with processed poultry feeds showed that ST1, ST4 and ST9 competed considerably in height and weight with birds fed with the commercial feed. The Cyanide content of the fresh cassava peel was 175mg/kg on day one which decreased to 0.00mg/kg in all treatments. Microbial contaminants present in the processed feeds include *Micrococcus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp. and *Aspergillus* sp. Total Heterotrophic Bacterial Count (THBC) ranged from 27.5×10^3 to 4.5×10^3 CFU/g while Total Fungal Count (TFC) ranged from 1×10^3 - 3×10^3 SFU/g. To this end, it is advisable that more research work on quality improvement of the feed and bioconversion of other waste be conducted.

Keywords: cassava peel, *Pleurotus ostreatus* and *Bacillus cereus*, poultry feed

1.0 INTRODUCTION

Poultry can include any commercialized and domesticated types of birds such as chickens, turkeys, pigeons, peafowl, ducks, geese, upland game birds (quail, pheasant, partridges etc) and ratites (ostriches, emu etc) grown for the production of eggs and meat for consumption and other purposes [1]. Poultry production has seen a great growth over the years due to a huge growth demand in meat production globally [2]. In 2007 pork meat, the dominant meat

32 source in Eastern Asia, declined in consumption, due to lack of supply, which led to a
33 plodding shift to poultry. In the Middle East, where competition with pork is non-existent, the
34 demand for poultry meat has also increased. In Nigeria from 1987 to 2002 poultry production
35 increased marginally from 175 to 200 million [3]. The poultry industry in Nigeria is currently
36 expanding in the past years, with a high increase of production since 2011. Hen egg
37 production in Nigerian as at 2011 was valued at \$527.49 million, ranking 19th in world first
38 in Africa [3].

39 Poultry farms are being industrialized for mass production and sometimes integrated with
40 ancillary businesses such as feed mill, breeders, hatchery, grow out farms, processing plants,
41 transportation and marketing [4]. Poultry feed include mash, pellets, crumbles and scratch
42 grain or chicken scratch, shell grit, medicated versus un-medicated, fermented feed, broiler
43 varieties [5]. USEPA (Environmental protection agency) stated in one of their publications on
44 [6] that Poultry feed can be produced on the poultry farm and fed through mills. Such feed
45 should consist of the energy giving substances, protein and micronutrient as maybe required.

46 Indiscriminate disposal of cassava peel; a common agro-waste in Nigeria; is an
47 environmental nuisance. Cassava wastes are usually found in the location of households and
48 factories where cassava roots are processed into garri, fufu, starch or flour. Cassava peel has
49 higher protein content than other parts of the root which can increase after proper treatments
50 with microorganisms (Ofuya and Nwajiuba, 1990, Tewe, 2004). Cassava peels can cause
51 environmental pollution but bio-treatment with microorganisms can successful reduces its
52 nuisance value in an environment (Aisien and Ikenebonhe, 2017). The poultry market in the
53 country is growing rapidly and the demand for affordable poultry feed is on the increase.
54 Poultry feed cost is hampering the expansion of the industry. Hence the need for cheap
55 locally made poultry feed. The study aimed at production of poultry feed through
56 biodegradation of cassava peel using *Pleurotus ostreatus* and *Bacillus cereus*.

57 2.0 MATERIALS AND METHODS

58 2.1 Sample Collection

59 Cassava peels were collected from Rumuokwachi, Obio/Akpor Local Government Area,
60 Rivers State, Nigeria. Samples were washed clean with tap water and sun dried for 3 weeks.
61 The cassava peel were ground to powder and packed in polythene bags for further use. Palm
62 press fiber collected from Rumuosi community and fish waste was collected from Ozuoba
63 Market were dried with hot air oven ground into powder. The soil sample was aseptically
64 collected from a dumpsite along Ozuoba /NTA road with the aid of a sterile bag and
65 conveyed to the laboratory for the isolation of *Bacillus cereus*.

66 2.2 Microbiological analysis

67 2.2.1 Culture media

68 *Bacillus cereus* was isolated from dump site by serial dilution and pour plate technique on
69 CM0617, a selective and diagnostic medium for enumeration of *Bacillus cereus*. Nutrient
70 Agar and Potato Dextrose Agar were used for the isolation and enumeration of bacterial and
71 fungal contaminants respectively. Media were sterilized by autoclaving at 121⁰C for 15mins
72 and was allowed to cool to about 45⁰C before use. Polymycin B supplement was added to the
73 CM0617 *Bacillus cereus* medium and Streptomycin (0.1%) was added to the Potato Dextrose
74 agar to prevent fungal and bacterial contamination of the media respectively.

75 2.2.2 Enumeration of Total Heterotrophic Bacteria Count (THBC)

76 This test was done to screen for the total viable aerobic, mesophilic and heterotrophic
77 bacteria present in each sample. The samples were serially diluted to 10⁻⁵ dilution using
78 physiological saline. Aliquots 0.1ml from 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions were spread aseptically
79 with a sterile glass rod on freshly prepared dry nutrient agar plates. The plates were incubated

80 at 37°C for 24 to 48 hours. After incubation, the TFC for each sample was recorded and
81 expressed in colony forming unit per gramme (cfug⁻¹).

82 **2.2.3 Enumeration of Total Fungal Count (TFC)**

83 This test was carried out to enumerate the total heterotrophic fungal species contained in the
84 treatment samples. Aliquots of 0.1ml from 10⁻¹, 10⁻² and 10⁻³ dilutions were spread
85 aseptically with a sterile glass rod on freshly prepared dry potato dextrose agar plates and
86 incubated at 28°C for 3-5 days. After incubation, the TFC for each sample was recorded and
87 expressed as spore forming unit per gram (sfug⁻¹).

88 **2.2.4 Purification and Characterization**

89 After incubation, distinct colonies observed on both the nutrient agar plates and potato
90 dextrose agar plates were isolated and purified by repeated sub-culturing. The bacterial
91 isolates were characterized using their macroscopic, microscopic and biochemical attributes.
92 The **bacterial** isolates were macroscopically examined for morphology and colony
93 characteristics and microscopically examined by gram staining and viewed under oil
94 immersion objective (x100 magnification). Biochemical test such as indole test, catalase test,
95 citrate test, motility test, urease test, starch hydrolyse test and sugar fermentation were carried
96 out as described by Fawole and Oso [7]. The isolates were identified using Bergey's manual
97 of systematic bacteriology Buchman and Gibbon [8]. **The fungal isolates were characterized**
98 **on the basis of their macroscopic and microscopic appearance with reference to standard**
99 **manual.**

100 **2.3 Collection and Tissue culture of *Pleurotus ostreatus***

101 The edible mushroom *Pleurotus ostreatus* was purchased from mushroom unit of
102 Agricultural demonstration farm at Choba Campus of the University of Port Harcourt, Port

Harcourt, Rivers State and the tissue of the mushroom was cultured on Potato Dextrose Agar media at 27°C for 72hrs (3days).

2.4 *Pleurotus ostreatus* spawning

Pleurotus ostreatus spawning was done using sterile spawn bottle according to the method of Fasidi and Kadiri [9]. The *Pleurotus* spawn was inoculated into the guinea corn grain and incubated. First guinea corn was washed in clean water, drained, transferred into spawn bottle, mixed with 5g of calcium carbonate (CaCO₃) and afterwards pasteurized before inoculation with 9mm mycelia disc per bottle (in triplicate) under aseptic condition and incubated at 27°C room temperature.

2.5 Biodegradation of Substrate (Cassava peel)

The substrate (cassava peel) was weighed to 150gram, sterilized by autoclaving and treated with 10ml, 20ml, 30ml of *Bacillus cereus*, *Pleurotus ostreatus* and a consortium of the two organisms (Table 1). The moisture contents of each weighed out substrates were increased by 60% (250mls). Biodegradation of substrate was for 28days in 500ml conical flasks.

Table1: Experimental setup

Sample treatment	Composition
ST1	150g cassava peel + 30 ml Consortium
ST2	150g cassava peel + 20 ml Consortium
ST3	150g cassava peel + 10 ml Consortium
ST4	150g cassava peel + 30 ml <i>Bacillus</i> sp.
ST5	150g cassava peel + 20 ml <i>Bacillus</i> sp.
ST6	150g cassava peel + 10 ml <i>Bacillus</i> sp.
ST7	150g cassava peel + 30 ml <i>Pleurotus ostreatus</i>
ST8	150g cassava peel + 20 ml <i>Pleurotus ostreatus</i>
ST9	150g cassava peel + 10 ml <i>Pleurotus ostreatus</i>

2.6 Proximate analysis of sample treatments

Moisture content was determined by the air oven method, carbohydrate determination was by Cleg Ansthron method, lipid was determined by Soxhlet extractor, ash determination was carried out using Loss on Ignition method, protein was determined by Kjeldahl method, and fiber determination was done by fiber by difference.

2.7 Hydrogen Cyanide Content Determination

Hydrogen cyanide content was determined through distillation and titration.

2.8 Fortification Process

Sample Preparation

A bag of palm press fiber was dried under high heat from bakery oven for a week. It crushed into powder form using grinding machine and autoclaved for 15 minutes at the temperature of 121°C. A bag of *Ethmalosa fimbriata* head was collected from Ozuoba market and was ground into powdery form, bagged in a clean polythene bag for further use.

Fortification procedure

- 10% of the fortification agent was required in each 100g of the sample treatments.
- 5g each of the palm press fiber and fish waste was measured out
- 90g of the sample treatments was measured
- The weighed fortification agents were added to the weighed sample treatments
- Samples were autoclaved for 15 minutes at the temperature of 121°C after fortification.

2.9 Feeding of a day old chicks processed feed and commercial product

Day old chicks were fed with the processed feed and commercial (hybrid) feed. Records of the size and weight of the chicks were taken to ascertain the growth rate in each monitoring

stage. A set of three birds for all treatment groups were feed and their weight, wing, neck and limb sizes determined at day 1, 14 and 28.

3.0 Statistical Analysis

The growth parameters for the different treatments were analyzed using one-way Analysis of Variance (ANOVA) with the SPSS vs 20 software.

3.0 Results and Discussion

3.1 Nutrient composition of palm press fiber and fish waste (*Ethmalosa fimbriata*)

The proximate for the nutrient composition of Palm Press Fiber (PPF) and Fish Waste (FW) is presented in Table 2. The result showed that lipid (45.24%) was the dominant nutrient in PPF and ash the least (1.45%). For FW, protein (41.56%) was the dominant nutrient and moisture the least (2.75%).

Table 2: The nutrient composition of palm press fiber and fish waste

Proximate composition	Concentration (% w/w)	
	Palm press fiber	Fish waste
Moisture	7.1	2.75
Ash	1.45	29.9
Lipid	45.24	7.88
Carbohydrate	3.35	6.33
Protein	14.44	41.56
Fibre	28.42	11.6

3.2 Nutrient composition before and after fortification

The result of the concentration of parameters before and after fortification is shown in Table 3. Moisture, ash, carbohydrate, lipid, protein and fiber in none fortified samples ranged from 82.7% to 77.53%, 6.7% to 4.4%, 24.03% to 8.83%, 4.9% to 2.1%, 17.5% to 10.94%, 69.24% to 49.77% respectively. Moisture, ash, carbohydrate, lipid, protein and fiber in the fortified samples ranged from 13.6 to 8.7%, 8.5% to 5.7%, 29.22% to 19.79, 8.5% to 5%, 20.25% to 14%, 43.3% to 28.05% respectively.

Table 3: Comparison of nutrient composition before and after fortification

Sample Treatment	NF/F	Moisture (%)	Ash (%)	CHO (%)	Lipid (%)	Protein (%)	Fibre (%)
1	NF	82.7	5.33	16.65	4.6	16.63	57.12
	F	12.8	8.5	21.12	8.5	19.31	29.77
2	NF	81.91	6.2	21.18	3.2	10.94	58.48
	F	11.4	7	24.18	5.1	14.88	37.44
3	NF	81	5.6	17.34	3.6	15.31	58.15
	F	11.8	6.9	19.79	8	18	35.51
4	NF	82	4.7	24.03	4.2	17.5	49.77
	F	11.7	5.7	28.25	6.7	20.25	30
5	NF	81.53	6.7	12.54	2.2	11.38	66.75
	F	8.7	7.8	21	5.2	14	43.3
6	NF	82	5.7	8.83	3.1	13.13	69.24
	F	13.6	7.2	22.87	5.6	16.25	34.48
7	NF	80.7	4.4	19.97	2.1	11.81	51.72
	F	11.8	6.9	23.91	5	14.44	37.95
8	NF	82.7	5.7	20.69	4.8	13.13	55.68
	F	11.4	5.9	29.22	6	14.88	32.6
9	NF	77.53	4.5	12.92	4.9	15.31	62.37
	F	11.3	6.9	28.72	7.9	18.13	28.05

NF=none fortified, F=fortified

3.3 Cyanide content of sample treatment

Result in Table 4 showed that the concentration of cyanide in the fresh cassava peel which was 175mg/kg reduced to within 15-50 mg/kg on day 7, 10-20 mg/kg on day 14 and completely absent in the final product (day 28).

Table 4: Cyanide level in sample treatments

Sample Treatment	Cyanide level		
	Day 7 (mg/kg)	Day14 (mg/kg)	Product (mg/kg)
ST1	50	10	NIL
ST2	40	10	NIL
ST3	25	10	NIL
ST4	30	10	NIL
ST5	25	20	NIL
ST6	20	10	NIL
ST7	15	10	NIL
ST8	50	10	NIL
ST9	20	10	NIL

3.4 Microbiological Characteristics of the different treatments

The results of the microbiological evaluation test after sterilization are presented in Table 5 and 6. The Total Heterotrophic Bacterial Count (THBC) ranged from 27.5 to 4.5 CFU/g while the Total Fungal Count (TFC) ranged from 1-3 SFU/g. The bacterial contaminants

were *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp. and *Micrococcus* sp., while the only fungal contaminant was *Aspergillus* sp. Sample treatment ST4 was free of bacteria contaminants; ST9 was free of fungal contaminants while ST5 was contaminant free.

Table 5: Bacterial and fungal counts after fortification

Sample Treatment	THBC ($\times 10^3$) cfug ⁻¹	TFC ($\times 10^3$) sfug ⁻¹
1	5	2
2	6.5	3
3	17	3
4	-	1
5	-	-
6	12.5	1
7	26	1
8	27.5	2
9	4.5	-

Table 6: Microbial contaminants

Sample Treatment	Bacteria	Fungi
1	<i>Micrococcus</i> sp.	<i>Aspergillus</i> sp.
2	<i>Staphylococcus</i> sp.	<i>Aspergillus</i> sp.
3	<i>Streptococcus</i> sp.	<i>Aspergillus</i> sp.
	-	<i>Aspergillus</i> sp.
5	-	-
6	<i>Bacillus</i> sp., <i>Staphylococcus</i> sp.	<i>Aspergillus</i> sp.
7	<i>Micrococcus</i> sp.	<i>Aspergillus</i> sp.
8	<i>Staphylococcus</i> sp.	<i>Aspergillus</i> sp.
9	<i>Staphylococcus</i> sp.	-

3.5 Growth parameters of chicks fed with processed feed and commercial product

Figure 1 show the weight, wing, leg and neck sizes of the bird from day 1 to 14

Figure 2 show the weight, wing, leg and neck sizes of the bird from day 1 to 28. The

weight of a day old chick was 0.1kg, size of the wing (2in), leg (2in) and neck (2in).

Results revealed a general increase in all the growth parameters for all birds

irrespective of the sample types with ST1, ST4 and ST9 showing comparable results to

the commercial feeds.

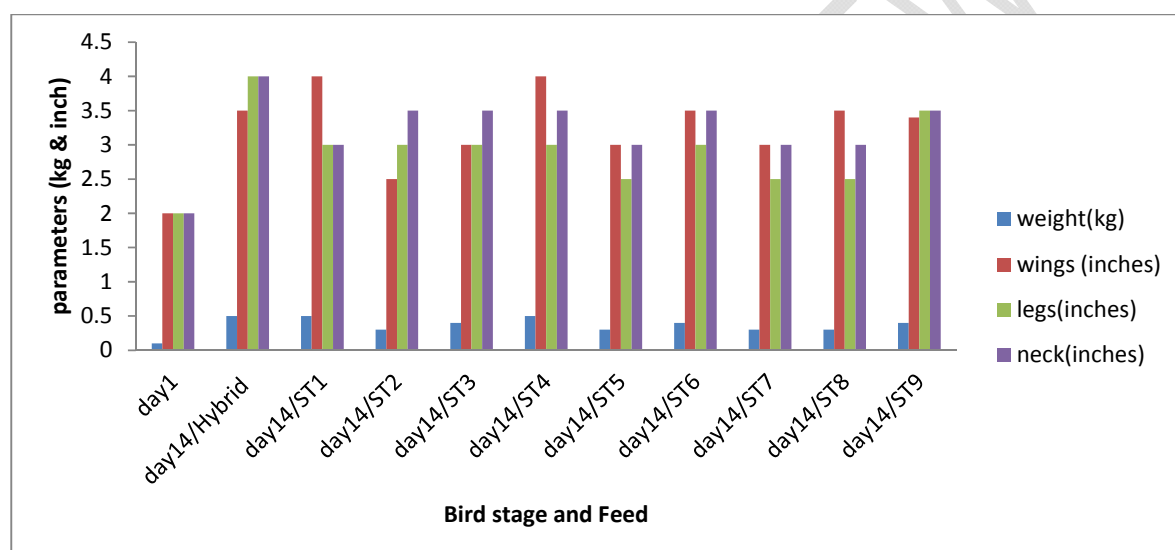


Figure 1: Parameters for determination of growth rate under the various treatments for day1 and 14

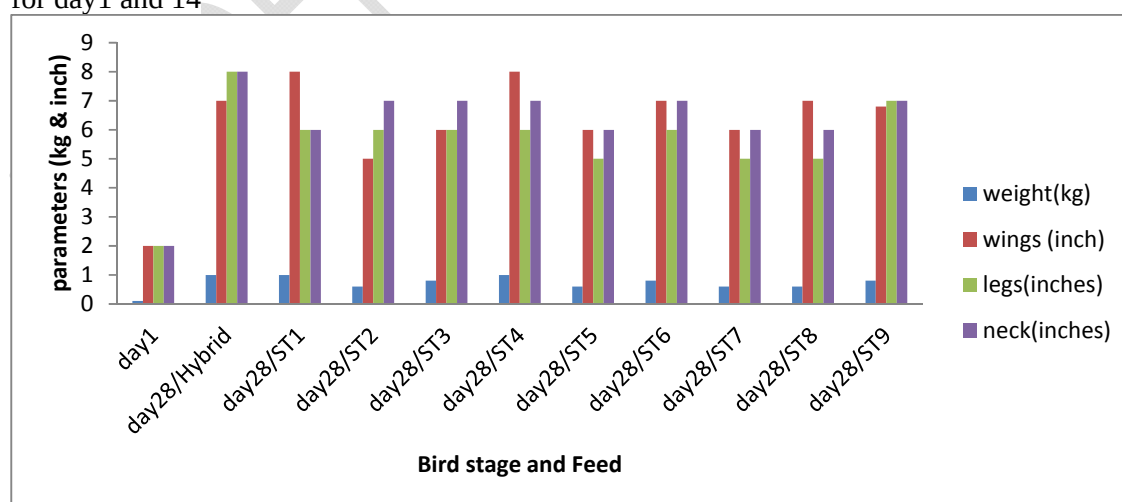


Figure 2: Parameters for determination of growth rate under the various treatments for day1 and 28

3.6 DISCUSSION

Results for the nutrient concentration of the palm press fiber and fish waste showed high lipid and protein levels, with low carbohydrate and moisture contents. Olapade and Meinday [10] reported that the percentage of fortification with bonga fish in bread baking increased the value of protein and other mineral. In comparison with the results from proximate analysis of Tilapia head (*Oreochromis mossambicus*) and bone flour by [11], the nutrient value of Bonga fish (*Ethmalosa fimbriata*) waste used in this study showed better levels in this fish than that of the tillapia.

The moisture contents of none fortified sample treatments were higher than the fortified. Morriss *et al.* [12] reported that removal of moisture by heat generally improves the concentration of nutrients, improve digestibility of food and also make some nutrients more available. Ash, carbohydrate, lipid and protein contents of the fortified sample were higher than in none fortified sample treatment. Microbial fermentation can increases the protein and fat contents of cassava peels due to the increase in the microbial mass, activities of lipolytic microorganisms to secrete extracellular enzyme (lipase), secretion of microbial oil into the fermenting medium and other products from the metabolism [13].

Antinutrient factors such as hydrocynic acid (HCN) is the key reason limiting the usage of cassava peel as animal feed [14]. Result showed that the concentration of cyanide in the fresh cassava peel was 175mg/kg. The determination of cyanide level of the product at the end of the biodegradation and fortification was 0.00mg/kg for all the sample treatments. The maximum levels of cyanide as established by WHO and Codex Alimentarius Commission is 10mg/kg and 2mg/kg for cassava flour and gari respectively [15], but in this study hydrogen cyanide was reduced to 0.00mg/kg through the biodegradation process employed.

Pathogenic microorganisms, mycotoxins and naturally occurring contaminants have effect on product safety [16] and cannot be ignored in this case. The Total Heterotrophic Bacterial Count (THBC) ranged from 27.5 to 4.5 CFU/g while the Total Fungal Count (TFC) ranged from 1-3 SFU/g. The microbial contaminants were *Aspergillus* sp., *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp. and *Micrococcus* sp., while the only fungal contaminant was *Aspergillus* sp. The processed feeds contained *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp. and *Micrococcus* sp. but not *E. coli*, *Salmonella* and *Listeria* which are capable of producing acute and chronic infections in birds [17]. The contaminant might be from the processing environment (Microbiology laboratory) or during handling.

Feeding of day old chicks involves administration of feed with the right amount of nutrients including vitamins, protein, lipids and carbohydrate [18]. Results revealed a general increase in all the growth parameters for all birds irrespective of the sample types with ST1, ST4 and ST9 showing comparable results to the commercial feeds. Statistical analysis showed no significant difference between treatments ST1, ST4, ST9 and the commercial feed.

Conclusion

Bio-processing of cassava peel by *Bacillus cereus* and *Pleurotus ostreatus* resulted in bioconversion of this agro-waste into useful end product, thereby freeing the environment of the pollutant. Upon fortification with palm press fiber and fish waste the biodegraded cassava peel was improved into a cyanide free poultry feed.

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