PROXIMATE AND MINERAL ASSAY OF MICROBE-FERMENTED CASSAVA TUBER WASTE IN DIFFERENT MIXING RATIOS AS A SUSTAINABLE FEED INGREDIENT IN LAYER'S FEED

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ABSTRACT
This study evaluates the nutritional enhancement of cassava tuber wastes through solid substrate fermentation with Aspergillus niger (ATCC: 16404). Three different mixing ratios of cassava peels (CP) and cassava stumps (CST) were used namely: 2/3 CST + 1/3 CP, 1/3 CST + 2/3 CP and 1/2 CST +1/2 CST. These different mixing ratios of CP and CST were microbially fermented with the candidate micro-organism for 5 days to obtain 2/3 MFCST + 1/3 MFCP, 1/3 MFCST + 2/3 MFCP and 1/2 MFCST +1/2 MFCST microbe-fermented (MF) value-added cassava tuber by-products designated Sample A, Sample B and Sample C respectively. They were assayed for proximate composition and mineral content in order to determine their nutritional quality for subsequent incorporation into layer diets. Moisture content in the three differently mixed cassava tuber by-products ranged ($p<0.05$) from 11.20 ±0.20% in Sample A to 15.20±0.20% in Sample C while the dry matter content in the three fermented samples differed ($p<0.05$) from 84.80±0.20% in sample C to 88.80±0.20% in Sample A. Samples A and C had the same value (81.80%) for NFE while Sample B differed significantly ($p<0.05$)from the two at 75.00±0.80%. Significant differences ($p<0.05$) was also observed in Ash content among the three samples. Significant difference was only observed in the Zinc content of the samples with 4.45±0.23ppm, 3.85±0.32ppm and 5.08±0.14ppm recorded for Samples A, B and C respectively. Conclusive, the three fermented cassava tuber wastes products are well balanced in the critical nutrients like Metabolizable energy, crude protein, fat and mineral elements that could meet the nutritional need of the egg laying species of livestock.
Keywords: Cassava tuber wastes, Fermentation, Feed ingredients, Mineral analysis, Proximate composition.

INTRODUCTION
The competition between human food needs and livestock feed requirements presents a significant global challenge, particularly in developing nations (Larisa, 2022). This competition is notably evident in the poultry industry, where conventional feed ingredients constitute approximately 70-80% of production costs (Makarynska and Vorona, 2024). Consequently, research has focused on alternative feed resources, particularly agro-industrial by-products, to ensure sustainable livestock production. Cassava *(Manihot esculenta*), with global production exceeding 276.7 million tonnes and Nigeria contributing 53 million tonnes annually, represents a promising alternative feed resource (Otekunrin and Sawicka, 2019). The crop's industrial processing generates substantial by-products, with peels and leaves constituting 25% of the whole plant biomass. These by-products contain bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial properties (Adetunji et al., 2015; Tzige and Herago, 2019). However, their utilization as livestock feed is limited by low crude protein content (1.3-3.5%), high fiber content (21-27.8%), and the presence of cyanogenic glycosides (Shahabuddin *et al*., 2024). Microbial fermentation has emerged as an effective biotechnological approach for improving the nutritional value of cassava by-products. Studies utilizing a consortium of microorganisms (*Lactobacillus delbrueckii, L. coryneformis, and Aspergillus fumigatus*) have demonstrated significant enhancement in protein content and reduction in anti-nutritional factors (Aro *et al*., 2010). Recent research indicates that incorporating 15% microbe-fermented cassava waste in poultry diets can reduce feed costs by 12% while maintaining production performance (Ogbuewu and Mbajiorgu, 2023). The objective of this study is to evaluate the potential of microbe-fermented cassava peels and stumps in different mixing ratios as alternative feed ingredients in poultry nutrition. This will be by investigating the proximate and mineral composition of microbe-fermented cassava peels and stumps of three different mixing ratios as possible feed ingredients for the commercial laying chickens.

MATERIALS AND METHODS
Experimental Site and Materials
The experiment was conducted in the Nutrition Laboratories of the Department of Animal Production and Health of the Federal University of Technology, Akure (7°10'N, 5°05'E), Nigeria. The research site is characterized by a humid tropical climate with an average annual rainfall of 1500mm and temperatures ranging from 22°C to 34°C. Fresh cassava peels (CP) and cassava stumps (CS) were obtained from the Arable Crops and Processing Section of the Federal College of Agriculture, Akure, Ondo State. Procedure of sterilization, inoculation and fermentation of the test materials were performed at the Department of Animal Production and Health Laboratories, while the mineral composition was done at Sustainable Research Laboratory, Akure.

Production of Microbe-Fermented Cassava Wastes

The microbe-fermented cassava tuber wastes were produced following the method described by Aro (2010). Fresh cassava peels (CP) and stumps (CST) were sun-dried for 3-5 days depending on environmental temperature and solar intensity. The dried materials were milled, packed in polythene sacks and stored in a dry place prior to microbial inoculation. The milled cassava peels and stumps were divided into three different samples each containing a mixture of the two test ingredients (the cassava peels and stumps) in the following mixing ratios: 2/3 CST + 1/3 CP, 1/3 CST + 2/3 CP and 1/2 CST +1/2 CST for Samples A, B and C respectively, each totaling 2,000g (2kg). For each sample, 2000ml of water was added to each 2kg of cassava waste mixture to obtain optimum water activity. The samples were steam-sterilized at 100°C for 30 minutes, cooled, and transferred to fermentation trays lined with transparent cellophane wrapper in a laminar flow chamber. Each 2kg sample was inoculated with 10ml of *Aspergillus niger* (ATCC: 16404) containing 2.5×10^5 spores/ml. The trays were covered and kept in a fermentation chamber for five days under ambient temperature and controlled humidity. The microbe-fermented samples were retrieved from the fermentation chamber on the fifth day and sun-dried for another five days to the required storage moisture content (10-13%) and stored in hermetically sealed nylon bags prior to proximate and mineral content determination.

Proximate Analysis

Proximate composition (moisture, ash, protein, fat, crude fibre, dry matter and NFE) of the three test samples was determined using standard AOAC (1990) while metabolizable energy was calculated from gross energy determined with Gallenkamp Ballistic Bomb Calorimeter.

Mineral Analysis

Mineral elements (Ca, P, K, Fe, Mg, Zn, Mn and Na) in the three samples were determined using atomic absorption spectrophotometer (Buck Scientific, 2004). Phosphorus was determined using the Vanado-molybdate method (AOAC, 1990).

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS version 23.0. Differences between treatment means were separated using Duncan's Multiple Range Test.

RESULTS

Proximate composition of microbe-fermented cassava peel and stump of different mixing ratios

The proximate composition of microbe fermented cassava peels and stumps of different mixing ratios is presented in Table 1. The mixing of the test ingredients at different ratios significantly ($p<0.05$) affected the proximate composition of the samples. Sample C exhibited the highest moisture content (15.20%), while Sample A had the lowest (11.20%). Conversely, Sample A has the highest dry matter content (88.80%), while Sample C showed the lowest (84.80%). Ash content was highest in Sample B (11.50%), which was significantly ($p<0.05$) different from the other two samples. Sample C had the lowest ash content (3.00%). Crude fibre (C.F.) was highest in Sample B (16.75%), although the difference was not statistically significant ($p=0.07$). Sample A had the lowest crude fiber content (8.00%). Crude protein (C.P.) levels were similar across samples, with no significant differences ($p=0.71$). Fat content was highest in Sample C (3.60%), notably higher than Samples A and B, although the difference was not statistically significant ($p=0.20$). Nitrogen-free extract (NFE) was highest in Samples A and C (both 81.80%), which were significantly different ($p=0.02$) from Sample B (75.00%). Metabolizable energy (M.E.) showed minimal variation across samples, ranging from 2750 in both A and C to 2780 in C, with no clear-cut trend related to the mixing ratios.

Mineral composition of microbe-fermented cassava peels and stump of different mixing ratios

The mineral composition of microbe fermented cassava peels and stumps of different mixing ratios is presented in Table 2. The mineral content analysis revealed significant variations only for zinc ($p<0.05$), with Sample C exhibiting the highest concentration (5.08 ppm). Other minerals showed no statistically significant differences among samples ($p>0.05$). Calcium level was numerically highest in Sample B (40.70 ppm) and lowest in Sample C (35.28 ppm). Phosphorus content was also highest in Sample B (43.50 ppm) and lowest in Sample C (33.90 ppm). Potassium showed a decreasing trend from Sample A to Sample C, with Sample A having the highest content (54.80 ppm) and Sample C the lowest (33.90 ppm), although this trend was not statistically significant ($p=0.07$). Iron, magnesium, manganese, and sodium levels were relatively consistent across all samples, with no significant differences observed.

Table 1: Proximate composition of microbe-fermented cassava peel and stump of different mixing ratios

Sample	Moisture	Dry matter	Ash	C.F	C.P	Fat	NFE	M.E
A	11.20±0.20^c	88.80±0.20^a	7.00±1.00^b	8.00±0.00	7.08±0.00	1.13±0.13	81.80±1.20^a	2750±0.00
B	13.50±0.30^b	86.50±0.30^b	11.50±0.50^a	16.75±2.75	7.00±0.00	1.98±0.02	75.00±0.80^b	2780±0.00
C	15.20±0.20^a	84.80±0.20^c	3.00±1.00^c	11.25±0.75	5.50±2.50	3.60±2.40	81.80±0.80^a	2750±0.00
SEM	0.74	0.74	1.60	1.77	0.72	1.07	1.50	6.32
P. Value	0.00	0.00	0.01	0.07	0.71	0.20	0.02	0.00

$^{a\ b\ c}$ = Means in the same row but with different superscripts are statistically (P<0.05) significant; C.F = Crude fibre; C.P = Crude protein; NFE = Nitrogen free extract; M.E= Metabolizable energy; A = 2/3 cassava stump + 1/3 cassava peel; B = 2/3 cassava peels + 1/3 cassava stump; C = 1/2 cassava peels + 1/2 cassava stump; SEM = Standard error of mean; P.Value = Probability value; Values are mean ± standard error of mean.

Table 2: Mineral composition of microbe-fermented cassava peels and stump of different mixing ratios

Sample	Ca	P	K	Fe	Mg	Zn	Mn	Na
A	39.53±6.25	36.48±2.34	54.80±2.99	2.18±0.32	55.53±3.89	4.45±0.23^{ab}	3.50±0.38	5.45±0.17
B	40.70±5.61	43.50±4.93	52.35±9.81	1.90±0.47	61.08±11.10	3.85±0.32^b	3.45±0.59	5.15±0.26
C	35.28±2.89	33.90±1.29	33.90±1.29	2.15±0.28	57.85±4.17	5.08±0.14^a	3.88±0.27	5.00±0.14
SEM	2.77	2.09	4.20	0.20	3.82	0.20	0.24	0.12
P.Value	0.74	0.15	0.07	0.85	0.83	0.02	0.76	0.37

^{a,b} = Means in the same row but with different superscripts are statistically (P<0.05) significant; Ca = Calcium; P = Phosphorus; K = Potassium; Fe = Iron; Mg = Magnesium; Zn = Zinc; Mn = Manganese; Na = Sodium; A = 2/3 cassava stump + 1/3 cassava peel; B = 2/3 cassava peels + 1/3 cassava stump; C = 1/2 cassava peels + 1/2 cassava stump; SEM = Standard error of mean; P. Value = Probability value; Values are mean ± standard error of mean.

DISCUSSION

The study investigated the proximate and mineral composition of microbe-fermented cassava peels and stumps of three different mixing ratios. The proximate analysis revealed that moisture content (15.20%) was highest in Sample C, this according to Wen *et al.* (2020) could promote mould growth and mycotoxin production at levels exceeding 14%. Ash content peaked at 11.50% in Sample B, with John *et al.* (2024) noting that high ash content might indicate soil contamination. The crude protein content (5.50-7.08%) showed no significant difference among the three test samples subjected to a monoculture of a selected micro-organism. Wang *et al*. (2024) suggested that microbial fermentation can improve protein quality despite low total protein. The values reported compared favourably with those reported by Aro and Aletor (2012) for cassava tuber wastes. Fat content was highest in Sample C (3.60%), though Bakare *et al.* (2012) cautioned about increased lipid oxidation risk during storage. Mineral analysis showed Sample C had the highest Zinc (5.08 ppm), which Zaslavskyi (2024) indicated could meaningfully contribute to livestock zinc requirements. Potassium decreased from Sample A to C (54.80 to 33.90 ppm), with Millena *et al*. (2022) noting that microbial fermentation can alter mineral bioavailability.

CONCLUSION

The study revealed that fermentation of cassava waste using *Aspergillus niger* (ATCC:16404) significantly improved the nutrient composition of the three cassava tuber waste samples, with optimal results at ⅔: ⅓ peel-to-stump ratio. Additionally, the three mixing ratios used under the current study balanced the energy levels of the wastes in equable proportion while the selected fungal strain concomitantly beefed up their energy contents. All the three samples showed higher digestible carbohydrate (NFE), protein, ash, mineral content than what are ordinarily obtained when the peels or the stumps are singly analysed for these particular nutrients. The result obtained in this study, thus reveal the potentials of this fermentation protocol and mixing ratios at enhancing the nutritive quality of these cassava tuber wastes.

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