PHYTOCHEMICAL PROFILING OF MICROBE-FERMENTED CASSAVA TUBER WASTE AT DIFFERENT MIXING RATIOS AS SUSTAINABLE FEED INGREDIENTS IN LAYER'S FEED

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ABSTRACT
The phytochemical profiling of three prospective livestock feed ingredients for layer is reported in this study. Cassava peels (CP) and stumps (CST) at three different mixing ratios were microbially fermented through the selective deployment of a candidate saprophytic fungus - Aspergillus niger (ATCC: 16404) in a fermentation protocol that lasted for 5 days. The three mixing ratios employed were: 2/3 CST + 1/3 CP, 1/3 CST + 2/3 CP and 1/2 CST +1/2 CST that produced 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. The quantitative screening of the phytochemicals in these samples showed the presence ($p<0.05$) of bioactive compounds including alkaloids (1.21-1.25 mg/g) in Sample C to A, saponins (1.35-1.61 mg/g) in Sample B to A and phytate (1.35-1.10mg/g) in Sample A to C. The cyanide content of these samples ranged ($p>0.05$) from 0.10mg/g in Sample C to 0.22mf/g in Sample B. The qualitative screening revealed the presence of alkaloids, saponins, cyanide, tannin, oxalates and phytate in all the three test samples. It can be concluded that these value added cassava tuber wastes contained appreciable amount of functional phytochemicals that qualify them as probable alternative feed resources in standard layers' ration formulation.
Keywords: Cassava peels, Cassava stumps, Feed ingredients, Fermentation, Phytochemicals.

INTRODUCTION

The nutritional roles of the phytochemical constituents of the feeding stuff cannot be overemphasized. Phytochemicals are biologically active groups of plant derived chemicals also known as secondary metabolites with inherent properties to prevent or to fight some common diseases (Oikeh *et al*., 2016). The easiest, cheapest and fastest way of identifying secondary metabolites present in any plant material of feeding stuff is through phytohemical screening (Geetha *et al*., 2014). Some of the phytochemicals that have been identified in this way include alkaloids, phytates, saponins, oxalates, cyanide and tannin.

Literature is replete with reports that confirmed the presence of these phytochemicals in cassava products and by-products like the pulp, leaves, peels and stumps (Aro and Aletor, 2012; Animashahun *et al*., 2022) with potential nutritional and health benefits to the users of such cassava products including both man and his livestock. Phytochemicals are known to possess antioxidant, anti-inflammatory, anti-microbial, anti-cholesterolemic and anti-cancerous properties. Phytochemical constituents of cassava products and by-products can be used to develop functional foods, nutraceuticals and pharmaceuticals. The objective of this study is to evaluate the potentials of microbe-fermented cassava peels and stumps at different mixing ratios as alternative feed ingredients in poultry nutrition by screening for their phytochemical constituents.

MATERIALS AND METHODS

Experimental Site and Materials

The experiment was conducted in the Department of Animal Production and Health of the Federal University of Technology, Akure (FUTA), 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 34C. 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, Nigeria. The phytochemical screening was done at the Functional Foods and Nutraceutical Laboratories of the Department of Biochemistry, Federal University of Technology, Akure, Nigeria.

Production of Microbe-Fermented Cassava Wastes

The microbially fermented cassava tuber wastes were produced following the method described by Aro (2010). Fresh cassava peels (CP) and cassava stumps (CST) were sun-dried for 3-5 days depending on environmental temperature and solar intensity. The dried materials were crushed, packed (in polythene sacks) and stored in a cool place prior to microbial inoculation. For each treatment samples, 2000ml of water was added to each 2kg of cassava waste mixture: *2/3 CST + 1/3 CP, 1/3 CST + 2/3 CP and 1/2 CST +1/2 CST*. The samples were

steam-heated at 100°C for 30 minutes, cooled, and transferred to fermentation trays lined with 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 under solid substrate fermentation. The fermented samples were "harvested" after five days of fermentation, sun-dried to appropriate moisture content and stored in a cool dry place prior to phytohemical screening.

Phytochemical Analysis

Quantitative and qualitative phytochemical screening were conducted according to standard methods of analyzing for saponins, tannins, alkaloids, oxalates, phytate and cyanide content in feeding stuffs (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 of the same statistical package.

RESULT

Quantitative screening of phytochemicals of microbe fermented cassava peel and stump of different mixing ratio

The result of the quantitative screening of phytochemicals of microbe-fermented cassava peel and stump of different mixing ratio is presented in Table 1. The parameters evaluated were significantly ($p<0.05$) influenced except cyanide, tannin and oxalate. The highest ($p<0.05$) values (1.25 mg/g) of alkaloid was observed in Sample A (2/3 cassava stump 1/3 cassava peel) while the least value (1.21 mg/g) was obtained in sample C (1/2 cassava stump 1/2 cassava peel). Sample A (2/3 cassava stump 1/3 cassava peel) recorded the highest ($p<0.05$) value of saponin (1.61 mg/g) while Sample B (2/3 cassava peel 1/3 cassava stump) had the least (1.35 mg/g). Cyanide ranged from 0.10 (Sample C) to 0.22 mg/g (Sample B). Sample A (2/3 cassava stump 1/3 cassava peel) recorded the highest value (0.91 mg/g) of oxalate while Sample B (2/3 cassava peel 1/3 cassava stump) had the least (0.70 mg/g). Tannin ranged from 0.01 (Sample A and B) to 0.13 mg/g (Sample C). The highest ($p<0.05$) values of phytate (1.35 mg/g) was observed in Sample C (1/2 cassava stump 1/2 cassava peel) while the least value (1.10 mg/g) was obtained in Sample A (2/3 cassava stump 1/2 cassava peel).

Table 1: Quantitative screening of phytochemicals of microbe fermented cassava peel and stump of different mixing ratio

Parameters (mg/g)	Sample A	Sample B	Sample C	±SEM	P-value
Alkaloid	1.25^a	1.24^a	1.21^b	0.02	0.008
Saponin	1.61^a	1.35^c	1.52^b	0.04	0.001
Cyanide	0.15	0.22	0.10	0.03	0.253
Tannin	0.01	0.01	0.13	0.03	0.119
Oxalate	0.91	0.70	1.30	0.09	0.234
Phytate	1.10^c	1.25^b	1.35^a	0.04	0.001

^{abc}= means significant ($p<0.05$) differences within row. Sample A = 2/3 cassava stump 1/3 cassava peel; Sample B = 2/3 cassava peel 1/3 cassava stump; Sample C = 1/2 cassava stump 1/2 cassava peel.

Qualitative Screening of phytochemicals of microbe-fermented cassava peel and stump of different mixing ratio

The qualitative screening of phytochemicals of microbe fermented cassava peel and stump of different mixing ratio is presented in Table 2. The phytochemical screening revealed the presence of alkaloids, saponins, cyanide, oxalate, phytate and tannins in all the samples.

Table 2: Qualitative Screening of phytochemicals of microbe-fermented cassava peel and stump of different mixing ratio

Parameters	Sample A	Sample B	Sample C
Saponin	+	+	+
Tannin	+	+	+
Alkaloid	+	+	+
Cyanide	+	+	+
Oxalate	+	+	+
Phytate	+	+	+

Key (+) = present; Sample A = 2/3 cassava stump 1/3 cassava peel; Sample B = 2/3 cassava; peel 1/3 cassava stump; Sample C = 1/2 cassava stump 1/2 cassava peel.

DISCUSSION

The quantitative and qualitative analysis of phytochemicals in microbe-fermented cassava peel and stump mixtures revealed significant variations in the concentrations and availability of certain bioactive compounds across different ratios. Phytochemicals are non-nutritive plant chemicals possessing varying degrees of disease- preventive properties and are beneficial to human as well as animal health. They are invaluable sources of raw materials for both traditional and orthodox medicine

In this study, alkaloid content showed significant variation among the samples. The highest concentration of alkaloids (1.25 mg/g) was observed in Sample A whereas the lowest concentration (1.21 mg/g) was found in Sample C. This suggests that a higher proportion of cassava stump may enhance the alkaloid content, potentially increasing the medicinal benefits of the mixture. Alkaloids play a key role in the defense mechanisms of plants, which might explain their relatively higher levels in cassava stumps, as they are more structurally robust in the stumps than the peels (Akinyele *et al*., 2017). The values reported in this study compared favourably with the values reported by Aro and Aletor, (2012) for cassava tuber wastes.

Saponins, known for their cholesterol-lowering and immune-boosting properties (Jolly *et al.,* 2024), were also significantly affected by the mixing ratios. The highest concentration was observed in Sample A. This finding indicates that the presence of cassava stumps can enhance saponin levels, likely due to their higher cell wall integrity, which may protect these compounds during fermentation as reported by Animashahun *et al.* (2022).
Cyanide is a well-documented anti-nutrient in cassava, with high levels posing health risks. However, fermentation typically reduces cyanide levels in cassava, making it safer for consumption. In this study, the cyanide concentration ranged from 0.10 mg/g in Sample C to 0.22 mg/g in Sample B. The slight variations in cyanide levels among the samples may be due to differences in the rate of cyanogenic glycoside breakdown during fermentation, influenced by the cassava mixing ratios. Sample C, with equal proportions of cassava stump and peel, had the lowest cyanide level, suggesting that this ratio may be more effective in reducing cyanide content through microbial fermentation. All the values reported were below the maximum tolerable level of cyanide (IITA, 1990).

CONCLUSION

Phytohemical profiling of the three microbe-fermented cassava tuber waste samples revealed the availability of some functional phytochemicals in the samples which can be harnessed for the formulation of functional feed for the egg type poultry species. The phytochemical screening showed reduced anti-nutritional factors, with notable decreases in cyanide (0.10-0.22 mg/g), tannins (0.01-0.13 mg/g), and oxalate (0.70-1.30 mg/g) levels across treatments. These findings suggest that while fermented cassava wastes holds promise as sustainable feed ingredients, addressing safety concerns is essential for their successful utilization in poultry nutrition.

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