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Research Article

Biological Enhancement and Compositional Characterization of Micro-Fungi Fermented Cassava Peels for Their Suitability as Poultry Feed Application

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Abstract

Cassava tuber processing generates significant waste, leading to environmental pollution. This study evaluates the enhancement of proximate, mineral, and antioxidant properties, alongside the reduction of antinutritional factors, in cassava peels via bio-fortification using solid-state fermentation (SSF) with microfungi. Peels were fermented separately with *Rhizopus oryzae*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. Results showed that *A. niger* fermentation yielded the highest carbohydrate (60.60%) and lowest fibre (7.99%), while *S. cerevisiae* produced the highest protein (14.71%) and ash (9.10%). *S. cerevisiae* treatment also maximized potassium and calcium levels, while *R. oryzae* significantly increased magnesium. Fermentation enhanced ferric reducing antioxidant power and DPPH radical scavenging, significantly elevating total phenolic content. Microbial action effectively degraded cyanide and cellulose compared to controls. These findings demonstrate that SSF significantly improves the nutritional and functional profiles of cassava peels, establishing them as a sustainable, low-cost alternative to maize in poultry feed formulations.

Keywords: Bio-fortification; Cyanogenesis; Broiler Nutrition; Nutritional Enrichment; Microbial Biotechnology

Abbreviations

SSF: Solid-State Fermentation; ANF: Anti-Nutritional Factors; DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid; FRAP: Ferric Reducing Antioxidant Power; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TEAC: Trolox Equivalent Antioxidant Capacity; AOAC: Association of Official Analytical Chemists; ASP: *Aspergillus niger* treatment; RHZ: *Rhizopus oryzae* treatment; SAC: *Saccharomyces cerevisiae* treatment; SEM: Standard Error of Mean; ANOVA: Analysis of Variance; AAS: Atomic Absorption Spectrophotometer.

Introduction

The survival and sustainability of the poultry industry in most developing countries will undoubtedly depend on its ability to secure a share of the limited food supply in competition with human consumption [1]. Despite the availability of numerous agricultural and agro-industrial by-products that could profitably be used as livestock feed, these resources are frequently underutilized [2]. Recent trends in poultry nutrition focus on the use of novel, non-conventional feeds with adequate nutritive values to substitute traditional ingredients like maize [3,4].

Cassava peel is a potential resource for animal feed, but its low protein content, high crude fiber, and elevated toxic cyanogenic compound levels limit its direct use [5]. Solid substrate fermentation (SSF) using various microorganisms presents a potential solution to increase its nutritional value and decrease toxic levels [6]. SSF is a biotechnological process where fungi, yeasts, and bacteria grow on the surface of solid organic substances without much water, acting as physical support for their development [6,7].

SSF enhances functional antioxidants, reduces anti-nutritional factors (ANFs) that interfere with nutrient absorption, and improves the bioavailability and digestibility of nutrients [8]. By breaking down complex organic materials into simpler compounds, SSF creates more nutrient-dense and functional materials. This paper aims to assess the use of fermented cassava peels to make them suitable for use in poultry feeds.

Materials and Methods

Cassava tubers and peels

Cassava tubers were collected from the vicinity of the Federal University of Technology, Akure, Nigeria, and validated by a crop scientist. The tubers were peeled, the peels were dried and ground with a hammer mill, while the tubers were grated into pulp for the inoculation of microorganisms.

Solid substrate fermentation technology

Ten fermenters were used in the process, three different fermenters for each organism, and one fermenter for the control.

Sample preparation

Following the process of Oboh [9], fresh cassava tubers were washed, peeled, and grated, after which the pulp was portioned into three distinct batches for independent fermentation. Each batch contained 1 kg of grated pulp, which was supplemented with a fungal inoculum and a prepared nutrient solution containing 8.0 g urea, 1.3 g KH_2PO_4, 11 g $MgSO_4$, and 2.0 g citric acid. The first batch was inoculated with *A. niger* (ASP), the second with *R. oryzae* (RHZ), and the third with *S. cerevisiae* (SAC). Fermentation was carried out for five days, after which the mash was mechanically pressed to recover all fermentation wastewater. The fermented pulp was then processed further into flour and garri.

Cassava peels obtained during processing were washed, dried, and milled. The ground peel was divided into three treatment groups and evenly spread on fermentation trays to an approximate thickness of 2 cm. Fermentation was initiated by applying the extracted cassava pulp wastewater, the same nutrient solution, and fungal inoculum, with one fungal strain assigned to each treatment group. The fermentation process was carried out for seven days, after which it was terminated, and samples were dried and collected for analysis. Non-microbe fermented cassava peels served as the control.

Proximate analysis

The Association of Official Analytical Chemists' established techniques were used for proximate analysis (AOAC 1995) [10].

Determination of moisture content

Powdered cassava peels of about 2 g were measured into a previously weighed crucible and dried in a hot-air oven at 105 °C for 4 hours until a constant weight was achieved. After drying, the samples were removed from the oven, cooled in a desiccator to prevent moisture absorption, and re-weighed. The difference in weight before and after drying was used to calculate the percentage moisture content following standard procedures described by AOAC (2005) [11].

Determination of ash content

Ash content was determined following a modified procedure described by Asouzu and Umerah [12]. Approximately 0.50 g of each cassava product sample was weighed into a pre-weighed porcelain crucible. The crucibles were placed in a muffle furnace and heated at 550 °C for 1 hour until complete combustion was achieved, indicated by the formation of a light gray to white ash. After ashing, the crucibles were removed from the furnace, cooled in a desiccator to avoid moisture absorption, and re-weighed. The percentage ash content was calculated based on the weight of the residue relative to the original sample weight.

Determination of crude fat

Crude fat content was determined using the Soxhlet extraction method as outlined by Asouzu and Umerah [12]. A 10 g portion of each ground peel was placed in a cellulose thimble and transferred into the Soxhlet extraction chamber. Extraction was carried out using n-hexane as the solvent, with the system heated at 35 °C for 3 hours to allow continuous solvent reflux. Upon completion, the solvent was evaporated, and the extracted lipid residue was

weighed. Crude fat content was expressed as a percentage of the original sample weight.

Determination of crude fibre

Crude fibre analysis was performed through successive acid and alkaline digestion. A 1.50 g sample of cassava peels was treated with 200 mL of boiling 1.25% sulfuric acid (H_2SO_4) and maintained at boiling for 30 minutes. The mixture was filtered by suction through poplin cloth and thoroughly rinsed with hot distilled water. The residue was then transferred into 200 mL of boiling 1.25% sodium hydroxide (NaOH) solution and boiled for another 30 minutes. Following filtration, the residue was washed sequentially with distilled water (once), methylated spirit (twice), and petroleum ether (three times). The cleaned residue was transferred into a previously oven-dried crucible of known weight and dried in an oven at 105 °C until a constant weight was obtained. The dried material was cooled in a desiccator, weighed, and subsequently incinerated in a muffle furnace at 450 °C for 3 hours. After cooling, the weight of the ash was recorded, and the crude fibre percentage was calculated from the loss in weight.

Determination of protein content

Crude protein determination was conducted using the micro-Kjeldahl digestion, distillation, and titration procedure. An oven-dried sample (0.50 g) was introduced into a 50 mL Kjeldahl digestion flask, followed by the addition of 10 mL concentrated sulfuric acid (H_2SO_4) and half a Kjeldahl catalyst tablet ($CuSO_4 \cdot 5H_2O$). The mixture was heated until a clear grey-white digest was obtained, after which heating continued for an additional two minutes to ensure complete digestion. The digest was allowed to cool and diluted to 100 mL with distilled water. A 10 mL aliquot of the digest was steam-distilled with 15 mL of 40% NaOH for 10 minutes into 20 mL of 2% boric acid solution containing three drops of mixed indicator in a 150 mL conical flask. The distillate was titrated against 0.01 M hydrochloric acid (HCl). Nitrogen content was calculated from the titre value, and crude protein content was obtained by multiplying nitrogen by a factor of 6.25. A reagent blank was analyzed concurrently to ensure accuracy.

Determination of carbohydrate

Total carbohydrate content was calculated by adding the total values of crude protein, crude fat, crude fibre, and total ash contents of the sample and subtracting it from 100% [12].

Lignin content

Using a modified method of Klason lignin assay, the cassava peels were treated with 72% sulfuric acid at room temperature for an hour. The sulfuric acid concentration was reduced to about 3%, and the sample was heated for a prolonged period (up to 4 hours) to complete the hydrolysis process. The residue was filtered, washed, dried, and weighed. The lignin content was then calculated based on the weight of the residue [13].

Cellulose content

Using the Updegraff method, the cassava peels sample was treated with an acetic acid-nitric acid mixture, and the mixture was then boiled for about 30 minutes. The remaining cellulose was collected by filtration and washed to remove the acids. The cellulose was then treated with sulfuric acid to break it down into glucose, which was measured using a colorimetric assay. The amount of glucose released was measured using a spectrophotometer, and the cellulose content was calculated based on this value [14].

Antioxidant analysis

Determination of total phenolic content

Total phenolic content of the aqueous peel extracts was quantified using the Folin–Ciocalteu colorimetric assay, following the procedure of Singleton and Rossi [15] with minor adjustments. Suitable dilutions of each extract, alongside a prepared gallic acid standard solution, were reacted with 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent, followed by neutralization with 2.0 mL of 7.5% sodium carbonate solution. The reaction mixtures were incubated at 45 °C for 40 minutes, after which absorbance readings were obtained at 765 nm using a UV-visible spectrophotometer.

Determination of total flavonoid content

Total flavonoid concentration was determined using a slightly modified aluminum chloride method as described by Meda., *et al*. [16]. Briefly, appropriately diluted extract samples were mixed with 0.5 mL methanol, 50 µL of 10% aluminum chloride, 50 µL of 1 mol L^{-1} potassium acetate, and 1.4 mL distilled water. The resulting mixture was allowed to stand at room temperature for 30 minutes, after which absorbance was measured at 415 nm. Total flavonoid content was calculated using quercetin as the reference standard.

DPPH free radical scavenging assay

The free radical scavenging capacity of each extract against DPPH radicals was evaluated based on the method of Gyamfi., *et*

al. [17]. A 50 µL aliquot of each diluted extract was combined with 550–600 µL of DPPH solution, and the mixture was incubated in darkness for 30 minutes to prevent photodegradation. Absorbance was subsequently recorded at 516 nm using a UV-visible spectrophotometer. Radical scavenging activity was calculated relative to a control containing all reagents except the sample.

$ABTS^+$ radical scavenging activity

ABTS radical scavenging activity was assessed according to the method described by Re., *et al*. [18]. Appropriately diluted extracts were transferred into test tubes and reacted with 1.8 mL of pre-formed $ABTS^+$ solution. The mixtures were incubated in a dark environment for 15 minutes, after which absorbance was measured at 734 nm. Antioxidant capacity was expressed as Trolox equivalent antioxidant capacity (TEAC) using Trolox as the standard.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed to determine the reducing potential of the extract samples based on a modified version of the method described by Benzie and Strain [19]. To each test tube, 100 µL of extract was added along with 150 µL of 0.2 M phosphate buffer and 250 µL of 1% potassium ferricyanide (KFeCN). The mixture was incubated at 50 °C for 20 minutes, followed by the addition of 250 µL of 10% trichloroacetic acid (TCA), 1 mL of distilled water, and 200 µL of ferric chloride ($FeCl_3$). Absorbance was read at 700 nm using a UV-visible spectrophotometer.

Ferrous Ion (Fe^{2+}) chelation assay

To assess iron-chelating activity, suitable dilutions of the extracts were introduced into reaction flasks, followed by the addition of 168 µL of 0.1 M HCl (pH 7.4) and 200 µL of 0.9% saline solution. The mixtures were diluted appropriately with distilled water and incubated at 37 °C for 15 minutes. Subsequently, 150 µL of freshly prepared $FeSO_4$ and 13 µL of orthophenanthroline were added. Absorbance was measured at 510 nm using a UV-visible spectrophotometer.

Determination of mineral elements

Mineral analysis was conducted following AOAC (1984, 1990) procedures [20,21]. Two grams (2 g) of the cassava peel sample were dry-ashed as previously described, after which 15 mL of 20% (v/v) nitric acid was added to dissolve the ash. The mixture was boiled, filtered through acid-washed Whatman No. 43 filter paper, and the residue rinsed three times with distilled deionized water into a 100 mL volumetric flask, then diluted to volume. Calcium, magnesium, iron, and zinc concentrations were determined using an atomic absorption spectrophotometer (Pye Unicam SP 9 AAS), while sodium and potassium were quantified using a flame photometer. Phosphorus determination involved pipetting 5 mL of sample extract into a 50 mL volumetric flask, adding distilled water and 10 mL of vanadomolybdate reagent, then measuring absorbance at 400 nm after standing for 10 minutes. Phosphorus concentration was obtained from a standard calibration curve.

Anti-nutritional composition analysis

Determination of phytate content

Phytate content was analysed using a modified method of Oyeyinka and Afolayan [22]. A 2 g sample was extracted with 2% hydrochloric acid for 3 hours in a 250 mL conical flask and filtered through Whatman No. 1 filter paper. An aliquot of 25 mL filtrate was mixed with 5 mL of 0.03% ammonium thiocyanate indicator and 53.5 mL of distilled water, then titrated against iron(III) chloride solution (0.00195 g Fe mL^{-1}) until a persistent brownish-yellow coloration appeared. Phytic acid percentage was calculated using the formula: % Phytic acid = Titre value × 0.00195 × 1.19 × 100.

Determination of cyanide content

Cyanide concentration was determined using a modified AOAC (1990) method [21]. A 4.00 g sample was soaked overnight in a mixture of 40 mL distilled water and 2 mL orthophosphoric acid. The sample was transferred to a distillation flask containing anti-foaming paraffin and anti-bumping chips, then distilled. Approximately 10 mL of distillate was collected into a receiving flask containing 40 mL of 0.25% NaOH. The distillate was diluted to 50 mL, after which 20 mL was titrated with 0.01 M silver nitrate following the addition of potassium iodide. Blank titrations were performed to a turbid endpoint for accuracy.

Determination of tannin content

Tannin concentration was determined using the method described by Makkar., *et al*. [23]. A 0.20 g sample was extracted with 10 mL of 70% acetone, placed in an ice-water bath to prevent solvent evaporation, and shaken for 12 minutes. The extract was cooled, filtered, and 0.5 mL of filtrate was mixed with 0.5 mL of distilled water, 0.5 mL of diluted Lowry reagent, and 2.5 mL of

20% sodium carbonate solution. After vortexing, the mixture was incubated for 40 minutes at room temperature, and absorbance was measured at 700 nm using a Corning colorimeter. Tannin concentration was calculated using a standard tannic acid calibration curve.

Statistical analysis

Graphical visualization and data plotting were performed using Python (Version 3.12) with the Matplotlib and NumPy libraries. Results are reported as mean ± SEM, with different superscript letters indicating significant differences among treatments ($p < 0.05$).

Results and Discussion

Proximate composition

Figure 1 shows the proximate composition of micro-fungi fermented cassava peels. There was a significant increase in the protein content of the micro-fungi fermented cassava peels with *S. cerevisiae* (14.71%) recording the highest value, followed by *R. oryzae* (RHZ) (14.55%), and *A. niger* (ASP) (13.20%) compared to the unfermented control (5.66%). The fibre content of all microbe-fermented peels significantly decreased compared to the control. Carbohydrate content slightly decreased for RHZ (54.58%) and SAC (51.26%), while ASP showed an increase (60.60%). Fat contents generally increased across most fermented products, while the cellulose content significantly reduced ($p < 0.05$) across the treatments.

Significant differences in moisture content among fermented samples reflected differences in fungal metabolic activities, with *S. cerevisiae* exhibiting the highest moisture retention and *A. niger* the lowest. Increased moisture content following fermentation improves palatability and digestibility, enhancing suitability for animal feeding [25]. Ash content increased across fermented treatments, particularly in *S. cerevisiae* and *R. oryzae*, indicating enhanced mineral retention and potential improvement in mineral bioavailability [25].

Fat content increased in all fermented samples, with *A. niger* showing the highest values, attributable to its pronounced lipolytic activity, which aligns with findings by Contesini., *et al*. [26] and supports increased dietary energy contribution valuable in livestock nutrition [27]. Crude fibre content decreased markedly across all fungal treatments, particularly in *R. oryzae* and *S. cerevisiae*, due to enzymatic degradation of cellulose and hemicellulose, as previously reported by Gaizauskaite., *et al*. [28]. This degradation enhances digestibility by releasing fermentable substrates through hydrolytic and oxidative enzymatic action [27].

Crude protein content increased significantly following fermentation, with *S. cerevisiae* recording the highest values due to microbial protein synthesis, corroborating Oboh [9]. This improvement is nutritionally important as it enhances the protein density of cassava peels. Lignin content remained largely unchanged, consistent with its structural role in plant tissues and resistance to microbial degradation [29]. Cellulose content declined post-fermentation, particularly in the *S. cerevisiae* treatment, thereby improving potential digestibility. Carbohydrate levels decreased in all fermented samples due to microbial utilization of starch as a carbon source for biomass synthesis [30], although *A. niger* showed less efficient utilization, aligning with Oboh [9].

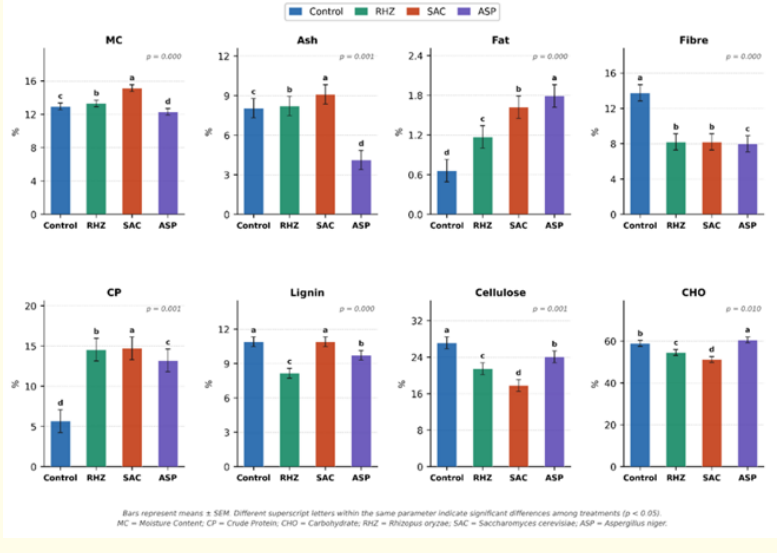

Figure 1. Proximate composition of micro-fungi fermented cassava peels.

Mineral composition

The mineral composition (Figure 2) of micro-fungi fermented cassava peels shows that the fermented peels did not exhibit a uniform general trend in mineral content. However, *S. cerevisiae* (SAC) exhibited the highest overall mineral ratio, with the highest values for sodium (111.1 ppm), potassium (123.2 ppm), and calcium (8.8 ppm). ASP ranked highest for iron (0.044 ppm) and magnesium (24.75 ppm), though ASP reached 31.15 ppm for magnesium.

Fermentation significantly altered mineral composition, with increases in sodium, potassium, calcium, iron, and magnesium depending on fungal species. Sodium and potassium were highest in *S. cerevisiae*, which may enhance feed palatability and support physiological functions such as osmotic balance and neuromuscular activity [31,32]. Iron content was highest in *A. niger*, beneficial for hemoglobin synthesis and animal health [33]. Zinc levels decreased in some fermented treatments, especially *S. cerevisiae*, necessitating further evaluation due to the mineral's role in immune and enzymatic functions [34], whereas calcium and magnesium enrichment support skeletal integrity and energy metabolism [35].

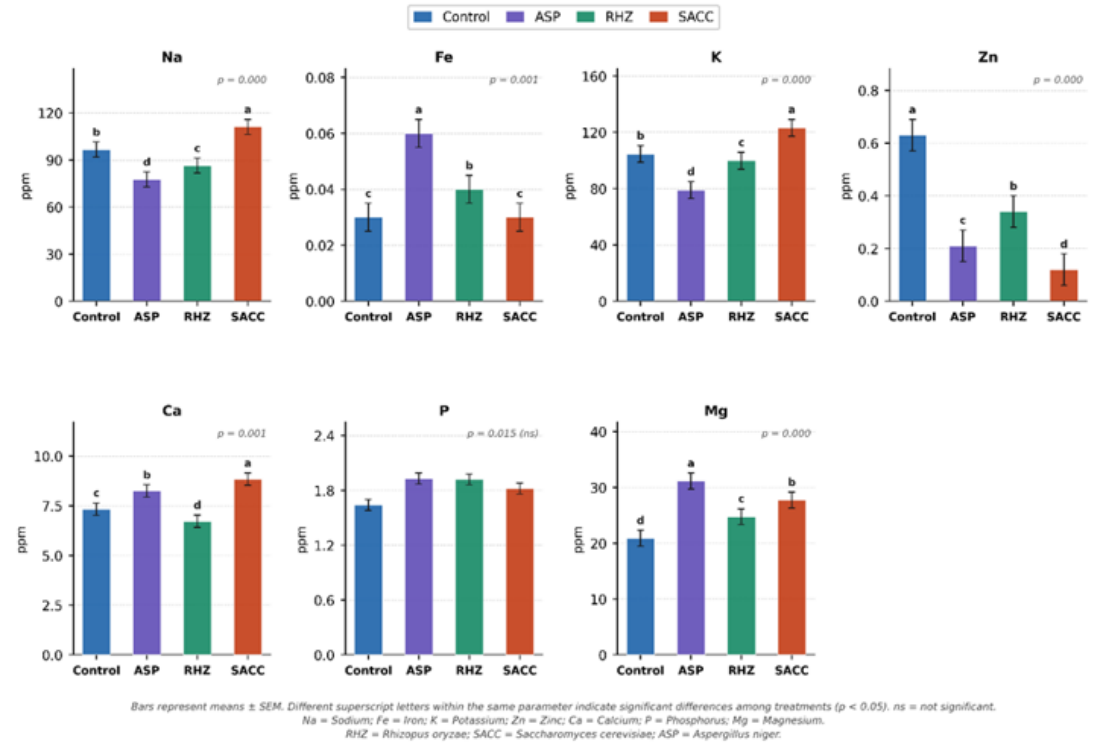

Figure 2: Mineral composition of micro-fungi fermented cassava peels.

Antioxidant composition

Figure 3 shows the antioxidant composition of cassava peels fermented with three different micro-fungal strains. Fermentation generally improved the antioxidant profiles. Peels fermented with RHZ and ASP showed a significant increase in phenolic content (248.7 and 227.7 mg g^{-1}, respectively) compared to the unfermented control (181.6 mg g^{-1}). While ASP and RHZ showed no increase in total flavonoid content (TFC), SAC caused a massive spike to 106.2 mg g^{-1}. Additionally, ABTS and DPPH radical scavenging abilities showed a steady rise in all microbe-fermented samples over the control, with SAC peaking in both. FRAP was also higher in the fermented samples, with RHZ ranking the highest.

Antioxidant analyses revealed that fermentation markedly enhanced the functional properties of cassava peels. Total phenolic content and FRAP values were highest in *R. oryzae*, indicating superior antioxidant capacity, while *S. cerevisiae* showed the highest flavonoid content and DPPH and ABTS radical scavenging activities, confirming improved free-radical neutralization potential [36,37]. Additionally, iron chelation capacity was highest in *R. oryzae*, which may reduce iron-induced oxidative stress [38].

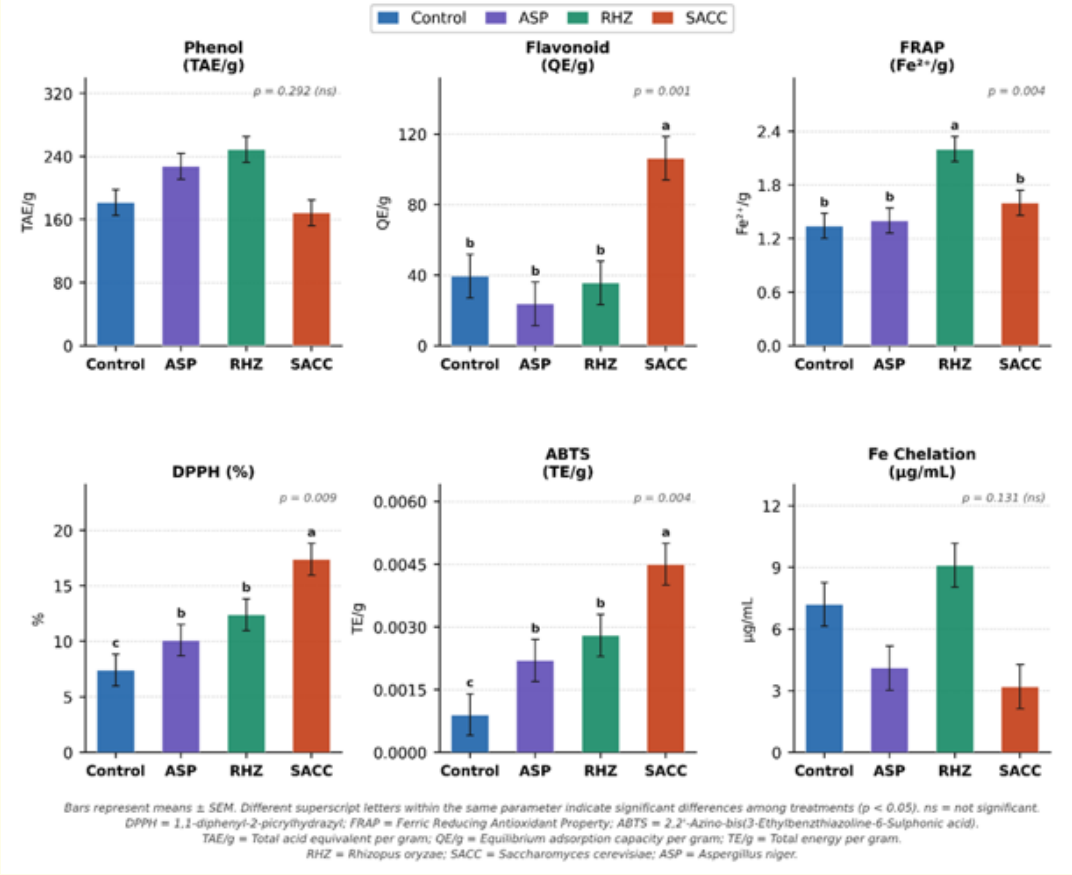

Figure 3: Antioxidant composition of micro-fungi fermented cassava peels.

Anti-nutritional composition

The anti-nutritional composition is shown in Figure 4. The cyanide content was dramatically reduced in the fermented samples (RHZ 0.01 mg g^{-1}, SAC 0.02 mg g^{-1}, ASP 0.02 mg g^{-1}) compared to the control (0.04 mg g^{-1}). There was also a significant decrease in the cellulose and lignin content of most micro-fungi fermented peels. Phytate decreased sharply in SAC (0.11 mg g^{-1}), while tannin showed a slight increase across all fermented groups compared to the control.

Anti-nutritional factors were significantly affected by fermentation. Tannin content increased in *S. cerevisiae*–fermented samples, potentially limiting nutrient utilization if uncontrolled [39], contradicting reductions reported by Hawashi., *et al*. [30]. Phytate content decreased notably in *S. cerevisiae*, enhancing mineral bioavailability [40], while cyanide content was lowest in *R. oryzae* fermented cassava peels, confirming the effectiveness of fermentation in detoxifying cassava residues [41].

Overall, these findings validate micro-fungal fermentation as a viable and sustainable strategy for improving the nutritional quality, antioxidant potential, and safety of cassava peels, supporting their effective use as alternative feed resources in livestock production systems [42].

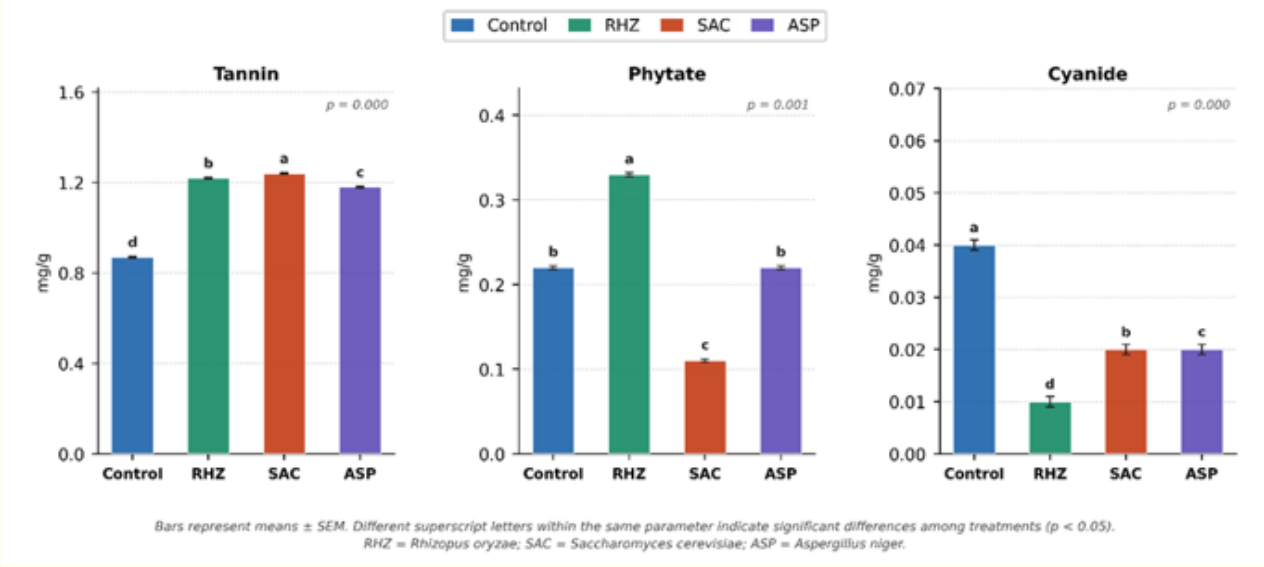

Figure 4: Anti-nutritional composition of micro-fungi fermented cassava peels.

Conclusion

Fermentation could dramatically improve the nutrient content of agro-industrial wastes and serve as an effective method for the pretreatment and eventual valorization of these wastes. To address the constraints facing the livestock feed industry, fermented cassava peel can be effectively used as a substitute for maize in broiler feed. This ensures that the supply of energy and other nutrients will no longer rely solely on maize, creating a low-cost, valorized waste alternative that minimizes environmental pollution and reduces the high cost of poultry production. Based on the results, micro-fungi fermented cassava peels can be confidently utilized as nutritional supplements or substitutes for conventional livestock feed; additional in vivo experiments should be conducted to assess their direct impact on the growth and overall performance of poultry birds.

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Conflict of Interest

The authors declare no conflict of interest.

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