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# Characterization of Fermented Cassava Flour from Cassava Grown in Nigeria

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## Abstract

Cassava is a major staple in tropical regions, yet its high carbohydrate content and inherently low levels of protein, essential amino acids, and micronutrients limit its nutritional adequacy. This study investigated the effect of controlled fermentation on the nutritional quality of cassava flour with the aim of identifying an optimal fermentation duration capable of enhancing its nutrient profile. Cassava flour samples were fermented for 5, 7, and 9 days and subsequently analyzed for protein content, amino acid composition, vitamins A and C, lipid concentration, and carbohydrate levels using standard analytical procedures. Results showed that 7-day fermentation produced the most significant improvements, yielding the highest protein content (9.93 mg/g), increased concentrations of vitamin C (1.31 mg/g) and vitamin A (0.15 mg/g), and enhanced essential amino acids such as leucine, lysine, and arginine. Carbohydrate content decreased progressively with fermentation, reflecting microbial utilization, whereas lipid content showed minimal variation across samples. Nutrient reductions observed after 9 days indicate prolonged fermentation. A 7-day fermentation period optimally enhances the nutritional value of cassava flour, offering a low-cost, scalable biofortification approach relevant to food security and nutrition interventions. These findings align with SDGs 2, 3, and 12 by supporting improved dietary quality, promoting better health outcomes, and encouraging sustainable processing practices. Further study is recommended to characterize the specific microorganisms responsible for the observed nutritional improvements and to support large-scale adoption in cassava-dependent communities.

**Keywords:** Cassava; fermentation; starch; characterization; nutrients; fibers

## 1. Introduction

Cassava (*Manihot esculenta Crantz*) is a major staple crop in tropical regions and sustains the diets of more than 500 million people globally (FAO, 2021). Its agronomic resilience, particularly its ability to thrive under drought, degraded soils, and low-input farming systems, has made it central to food security strategies across Nigeria (Prasad et al., 2024). Despite its widespread consumption and caloric value, cassava remains nutritionally inadequate: it contains minimal protein, low-quality amino acids, and insufficient concentrations of essential micronutrients such as vitamins A and C, iron, and zinc (Chen et al., 2024; Akpoghelie et al., 2025). Consequently, populations that rely heavily on cassava-based foods are disproportionately affected by micronutrient deficiencies and protein-energy malnutrition, often described as "hidden hunger" (Fernández-Tomé et al., 2023).

Fermentation is a longstanding processing technique used to enhance cassava safety and palatability, particularly through the reduction of cyanogenic glycosides and improvement of digestibility (Bamidele, 2025). Beyond detoxification, fermentation has gained recognition for its potential to modify the nutritional and biochemical characteristics of foods through microbial metabolism. Studies have shown that fermentation can influence amino acid release, modify carbohydrate structure, and contribute to the biosynthesis or retention of certain vitamins (Knez et al., 2023). However, existing evidence remains fragmented and inconsistent regarding how fermentation duration specifically influences nutrient enhancement in cassava flour. Most research has focused either on detoxification efficiency or sensory improvements, with limited systematic evaluation of how varying fermentation times modulate protein enrichment, amino acid profiles, and vitamin content. This represents a critical knowledge gap, given that fermentation time is one of the most controllable and scalable variables in low-resource settings.

Although cassava is widely used as a formal and informal food in Nigeria, empirical data that define an optimal fermentation timeline capable of achieving nutritional enhancement beyond basic detoxification are lacking (Bamidele, 2025). Without such evidence, cassava continues to contribute primarily to caloric sufficiency without adequately addressing micronutrient needs. This is essential for designing low-cost, culturally acceptable, nutrition-sensitive food processing interventions aligned with national and global strategies to reduce malnutrition. This study therefore provides a targeted nutritional characterization of cassava flour fermented for 0, 5, 7, and 9 days.

This study clearly established an evidence-based optimal fermentation duration that simultaneously enhances protein content, essential amino acids, and micronutrient concentrations. Unlike previous studies that treat fermentation primarily as a detoxification process, this study positions controlled fermentation as a natural, low-cost biofortification strategy capable of improving the nutritional quality of cassava flour without external fortificants or industrial additives. This distinction is significant for cassava-dependent populations and for stakeholders aiming to strengthen nutrition-sensitive value chains.

The findings contribute directly to food and nutrition security objectives by offering practical, scalable guidance relevant to household processors, small- and medium-scale cassava industries, and regulatory agencies. The study also complements national policy initiatives such as Nigeria's National Policy on Food and Nutrition and global WHO/FAO recommendations that promote food-based approaches to combat malnutrition (WHO/FAO, 2014).

### 2.1 Study Area and Raw Materials

Fresh cassava roots (variety TME 419), known for their suitability in flour production, were sourced from a local farmland in Oghara, Delta State, Nigeria (5°59'N, 6°10'E). The TME 419 cassava variety was specifically selected because it is an improved, high-yielding, and disease-resistant cultivar widely promoted in southern Nigeria for industrial flour production (Owoseni et al., 2021). Although regional preferences vary and some areas, such as parts of Kogi State, report less favorable sensory attributes, TME 419 remains one of the most stable varieties for flour yield, starch recovery, and processing consistency. Its low post-harvest deterioration and high dry matter content make it particularly suitable for fermentation and laboratory-scale flour characterization. The experimental work was conducted at Delta State Polytechnic, Otefe-Oghara, while subsequent sample characterization was carried out at the Institute for Agricultural Research, Ahmadu Bello University, Zaria. The study area experiences a tropical monsoon climate with annual rainfall of 2,000-3,000 mm, mean annual maximum temperature of 30-33 °C, and soils classified as sandy loam with acidic pH (4.5-6.0).

### 2.2 Equipment Used

All equipment used in this study was selected to ensure analytical precision, reproducibility, and compliance with internationally accepted laboratory standards. Fresh cassava tubers were processed using a 3.5 HP (Model: CMX-35, Chimezie Engineering Works, Nigeria) petrol-powered cassava grating machine for pulping, a mechanical hydraulic press (Model: HP-20T, Qiaolian Industrial Co., China) for dewatering, and a thermostatically controlled hot-air oven at 55 °C (Model: DHG-9140A, Memmert GmbH, Germany) for drying. Dried samples were milled with a laboratory hammer mill (Model: FZ102, Shanghai Tianhe Pharmaceutical Machinery Co., China) and sieved through a 0.25 mm stainless stell mesh (Model: ASTM E11-60, Endecotts Ltd., UK). Nutrient analyses were carried out using a UV-Visible spectrophotometer (Model: UV-1800, Shimadzu Corporation, Japan) for vitamin A and C determination as well as carbohydrate absorbance readings, Soxhlet extraction apparatus (Model: XT15, Alconox Scientific Instruments, UK) for lipid analysis, Kjeldahl digestion (Model: KDN-08C, Shanghai Xinjia Scientific Instruments Co., China) for total nitrogen and protein quantification, distillation, titration unit (for total nitrogen and protein estimation), flame photometer (Model: FP6410, Xylem Analytics, UK) for sodium and potassium determination, and atomic absorption spectrophotometer (AAS) (Model: AA-7000, Shimadzu Corporation, Japan) for calcium, magnesium, zinc, copper, and iron analysis. An Amino Acid Analyzer (Model: 120A PTH, Applied Biosystems, USA) was used for amino acid profiling, and all reagents used were of analytical grade (BDH, UK). Auxiliary

laboratory instruments included a water bath (Model: HH-4, Grant Instruments, UK), analytical balance (Model: Sartorius Entris 224-1S, Sartorius AG, Germany), , and rotary evaporator (Model: RE-52A, Yarway Tech, China). All reagents used were of analytical grade (BDH, UK), and calibration of instruments followed manufacturer specifications before each batch analysis to ensure accuracy.

### 2.3 Cassava Harvesting and Preparation

Cassava tubers were harvested 18 months after planting, peeled to remove the outer cortex and inner rind, and thoroughly washed to eliminate dirt and debris. The peeled tubers were grated into a fine mash using a 3.5 HP petrol-powered grating machine. Special attention was paid to maintaining the natural whiteness of the cassava throughout processing. Peeling, grating, fermentation, and drying were carried out promptly after harvesting to prevent enzymatic browning and ensure that the final flour met acceptable color standards in line with NAFDAC quality expectations for food-grade cassava flour. The mash was packed in sacks and left to ferment naturally for 5, 7, and 9 days. After fermentation, excess water was removed by mechanical pressing. It is important to note that the fermentation process employed in this study was a natural (spontaneous) fermentation without the addition of starter cultures, reflecting common local processing methods. The dewatering stage was carried out using a mechanical press immediately after fermentation to ensure adequate moisture reduction before oven-drying. Drying was performed at a controlled temperature of 55 °C for 24 h to prevent nutrient degradation and maintain flour color and texture. The dried samples were milled into fine flour, sieved (0.25 mm mesh), packaged in sterile polyethylene bags, and stored at 4 °C until analysis. Although the present study focused primarily on the nutritional enhancement of fermented cassava flour, detoxification remains a critical safety concern. Cassava contains cyanogenic glycosides (linamarin and lotaustralin), which can release hydrogen cyanide if not adequately processed. While cyanide quantification was not performed in this experiment, fermentation inherently aids detoxification by promoting enzymatic hydrolysis and volatilization of cyanide compounds (Ziemah et al., 2025). Future work will include total cyanide determination using the alkaline picrate method to complement the nutritional analysis.

### 2.4 Experimental Design

Unfermented cassava flour was used as control. Four sample categories were analyzed:

I. Unfermented (0 days)
ii. 5-day fermented flour
iii. 7-day fermented flour
iv. 9-day fermented flour

All experiments were conducted in triplicate to ensure the reliability, reproducibility, and statistical robustness of the analytical results. Triplicate measurements minimize the influence of random experimental error arising from sample heterogeneity, instrument variability, and environmental fluctuations during analysis. This approach aligns with standard laboratory practice in nutritional and food science research, where triplicate determinations are required to validate the precision and consistency of analytical outcomes.

### 2.5 Nutrient Characterization

The protein content was determined using the Kjeldahl digestion, distillation, and titration procedure, in accordance with AOAC (2006) official methods. Protein content was calculated using a nitrogen-to-protein conversion factor of 6.25. The amino acid profile was obtained after defatting the samples with a chloroform/methanol mixture (2:1, v/v), followed by hydrolysis with 6 N HCl at 110 °C for 24 hours. The hydrolysates were neutralized with NaOH, and the amino acid composition was quantified using an Applied Biosystems 120A PTH Amino Acid Analyzer with a C18 reverse-phase column, where peak areas were compared against authenticated standards for quantification. Total carbohydrate concentration was determined by the phenol-sulfuric acid method, using glucose as the reference standard, and absorbance was measured at 490 nm with a UV–V is spectrophotometer. The lipid content was assessed using Soxhlet extraction with petroleum ether (boiling point 60-80 °C) as the solvent, and the percentage of lipid was determined gravimetrically. Vitamin C content was quantified via the 2,6-dichlorophenolindophenol (DCPIP) spectrophotometric method, with absorbance measured at 760 nm. Vitamin A was determined after extracting the samples in isopropanol and incubating overnight, followed by absorbance measurement at 326 nm using a UV-Vis spectrophotometer. For elemental analysis, samples were digested using a nitric–sulfuric acid mixture, with sodium and potassium concentrations determined by flame photometry, while calcium, magnesium, zinc, copper, and iron were analyzed by atomic absorption spectroscopy (AAS).

### 2.6 Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) to determine the effect of fermentation duration on nutrient composition.

## 3. Results and Discussion

### 3.1 Protein Content

Fermentation significantly influenced the protein content of cassava flour. As shown in Table 1, protein levels increased from 7.49 mg/g in the unfermented control to 8.11 mg/g after 5 days and peaked at 9.93 mg/g on day 7. This enhancement is consistent with reports that microbial proliferation during fermentation can increase crude protein through microbial biosynthesis and enzymatic breakdown of macromolecules into simpler nitrogenous compounds (Morgan & Choct, 2016). The sharp decline to 5.72 mg/g after 9 days suggests nutrient depletion and potential proteolysis under extended fermentation, indicating that beyond a threshold, microbial activity begins to degrade rather than enrich protein. These findings firmly establish 7 days as the optimal fermentation duration for protein enrichment in cassava flour.

Table 1: Protein content of cassava flour across fermentation periods

| Fermentation period (days) | Protein (mg/g) |
|---|---|
| 0 (Unfermented) | 7.49 ± 0.22 |
| 5 | 8.11 ± 0.18 |
| 7 | 9.93 ± 0.31 |
| 9 | 5.72 ± 0.25 |

### 3.2 Amino Acid Profile

Fermentation also influenced the amino acid composition of cassava flour, with the 7-day sample exhibiting the highest concentrations of essential amino acids (Table 2). Leucine (2.60 g/100 g protein), lysine (1.72 g/100 g protein), and arginine (3.36 g/100 g protein) were most abundant after 7 days of fermentation, confirming that microbial metabolism during this period promotes amino acid release and possibly de novo synthesis. The enhanced levels of non-essential amino acids such as glutamic and aspartic acids further demonstrate improved protein quality, as these amino acids play key metabolic roles in nitrogen transport and energy production (Idrees et al., 2020). The reduction observed on day 9 reflects the onset of amino acid degradation processes such as deamination and decarboxylation, frequently associated with prolonged microbial activity. These results showed the importance of carefully controlled fermentation duration to maximize nutritional benefits.

Table 2: Amino acid composition of cassava flour at different fermentation periods (g/100 g protein)

| Amino Acid | 0 days | 5 days | 7 days | 9 days |
|---|---|---|---|---|
| Leucine | 1.88 | 2.10 | 2.60 | 1.92 |
| Lysine | 1.20 | 1.40 | 1.72 | 1.10 |
| Arginine | 2.55 | 2.89 | 3.36 | 2.40 |
| Glutamic acid | 3.55 | 4.10 | 4.62 | 3.20 |
| Aspartic acid | 2.20 | 2.60 | 3.01 | 2.05 |

Non-essential amino acids like glutamic acid and aspartic acid were also most abundant in the 7-day fermented sample. These amino acids play important roles in nitrogen metabolism and energy production (Idrees et al., 2020).

### 3.3 Vitamin Content

Vitamins A and C increased substantially during fermentation, with the highest concentrations recorded on day 7, 0.15 mg/g for vitamin A and 1.31 mg/g for vitamin C (Table 3). These results align with studies showing that controlled fermentation enhances vitamin availability due to microbial synthesis, enzymatic release of vitamin precursors, and improved matrix breakdown (Campos et al., 2025). The low unfermented flour (0.08 mg/g vitamin A; 0.39 mg/g vitamin C) highlight the naturally limited micronutrient profile in cassava. Beyond 7 days, both vitamins declined, due to oxidative degradation and microbial utilization. This pattern reinforces that extended fermentation is counterproductive to micronutrient preservation. The observed peak at 7 days therefore represents a critical turning point for designing fermentation protocols aimed at micronutrient enhancement. These findings align with previous studies reporting that fermentation enhances water-soluble vitamin availability but prolonged fermentation results in degradation (Dhiman et al., 2025).

Table 3: Vitamin composition of cassava flour during fermentation (mg/g)

| Fermentation period (days) | Vitamin A | Vitamin C |
|---|---|---|
| 0 (Unfermented) | 0.08 ± 0.01 | 0.39 ± 0.05 |
| 5 | 0.12 ± 0.02 | 0.82 ± 0.08 |
| 7 | 0.15 ± 0.02 | 1.31 ± 0.11 |
| 9 | 0.10 ± 0.01 | 0.61 ± 0.07 |

### 3.4 Carbohydrate and Lipid Content

Carbohydrate content decreased progressively during fermentation, from 120.69 mg/g in unfermented cassava to 58.99 mg/g after 7 days (Table 4). This reduction reflects microbial utilization of carbohydrates as a primary energy source during fermentation. The slight increase to 62.66 mg/g on day 9 may indicate reduced microbial demand as substrate availability diminishes or the formation of intermediate carbohydrate derivatives. Lipid content remained relatively

consistent across samples (0.52-1.12%), which is expected given cassava's inherently low fat content and the limited effect of fermentation on lipid metabolism (Bamidele, 2025). These findings confirm that fermentation mainly affects protein, amino acids, and micronutrients rather than altering the lipid fraction in cassava.

Table 4: Carbohydrate and lipid content of cassava flour during fermentation

| Fermentation period (days) | Carbohydrate (mg/g) | Lipid (%) |
|---|---|---|
| 0 (Unfermented) | 120.69 ± 2.5 | 0.52 ± 0.03 |
| 5 | 92.35 ± 1.9 | 0.83 ± 0.04 |
| 7 | 58.99 ± 1.6 | 1.12 ± 0.05 |
| 9 | 62.66 ± 1.8 | 1.05 ± 0.04 |

### 3.5 Implications for Food Security and Nutrition

The results demonstrate that a 7-day fermentation period optimizes the nutritional profile of cassava flour. This balance between detoxification, protein enrichment, and micronutrient enhancement supports role of cassava as a vehicle for combating malnutrition in cassava-dependent populations. The improvements in lysine and vitamin A are particularly significant, as deficiencies in these nutrients remain widespread in sub-Saharan Africa (Zhao et al., 2022). It is important to note that beyond nutritional improvement, fermentation also contributes to cassava detoxification by reducing cyanogenic glycosides to safe levels suitable for human consumption. Although this study did not quantify residual cyanide, literature indicates that properly fermented cassava products generally fall within the safety limit of NAFDAC ( Ziemah et al., 2025). Future studies will integrate cyanide assays to substantiate the consumptive safety of the optimized fermentation duration.

In addition to nutritional enhancement, product quality remains essential for regulatory compliance and consumer acceptance. The whiteness of cassava flour is a critical NAFDAC requirement, as discoloration often indicates delayed processing or contamination. The immediate peeling, fermentation, and drying approach adopted in this study not only improved nutrient retention but also produced a uniformly white flour suitable for safe human consumption and potential regulatory approval.

## 3. Conclusion

This study demonstrated that fermentation significantly alters the nutritional composition of cassava flour, with a clear improvement in protein content, amino acid profile, and vitamin concentrations at an optimal fermentation duration of 7 days. At this stage, protein content peaked at 9.93 mg/g, accompanied by marked increases in essential amino acids such as lysine, leucine, and arginine. Similarly, vitamins A and C were significantly enhanced, reaching 0.15 mg/g and 1.31 mg/g, respectively. Beyond 7 days, nutrient levels declined, emphasising the importance of controlling fermentation duration to avoid over-fermentation and subsequent nutrient degradation. The findings have important policy implications. Optimizing fermentation practices can contribute to addressing protein-energy malnutrition and micronutrient deficiencies in cassava-dependent populations. This aligns with the Nigerian National Policy on Food and Nutrition (2016) and global WHO/FAO strategies that advocate for food-based interventions to improve dietary quality. Standardized fermentation protocols can help ensure that locally consumed cassava products contribute more effectively to nutritional security. Agencies including the National Agency for Food and Drug Administration and Control (NAFDAC) could adopt these insights to establish benchmarks for fermented cassava flour, ensuring both safety and nutritional adequacy.

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## Conflict of Interests

Omidih, Avuwha Lucksyn wishes to state on behalf of the other authors that there is no conflict of interests.