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Effect of Different Fermentation Times on the Nutritional Value and Microbial Load of Cassava (*Manihot esculenta*) Flour

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Abstract

Fermentation is used as a process to reduce the level of hydrogen cyanide from cassava roots. This study aimed at evaluating the effect of different fermentation times on the nutritional value of cassava flour, *Manihot esculenta*. Cassava tubers were bought from Bokkos Main Market, processed at the National Veterinary Research Institute Vom, into flour using standard methods, and analyzed for microbial load, nutrient composition and hydrogen cyanide level at different fermentation times (0hours, 24 hours, 48 hours, and 72 hours). The results showed that fermentation time significantly reduced the hydrogen cyanide levels from 0.024mgHCN/10g at 0hours of fermentation to about 0.004mgHCN/10g by 72hours and that increased fermentation time impacted microbial succession with decreased pH from 7.4 to about 4.3 with bacterial and fungal counts peaking at 48 hours and decreasing by 72 hours. Nutritional analysis demonstrated improved nutrient profiles with prolonged fermentation, indicated by an increase in crude protein, crude fibre, and lipids with reductions in anti-nutritional factors which enhanced safety of the final product. Optimal fermentation was identified at 72 hours for balancing nutritional value with microbial safety and establishing that increased fermentation time significantly improved the nutrients profile of the fermented cassava flour.

Keywords: Fermentation time, Nutritional value, Hydrogen cyanide and Microbial load

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Introduction

Cassava (*Manihot esculenta*) is a vital crop in numerous tropical countries, serving as a cost-effective source of energy for millions of people, particularly in sub-Saharan Africa (Kawano, 2023). Globally, it ranks the fourth most significant energy-producing crop, following rice, sugarcane, and maize (Burns *et al.*, 2010). Notably, cassava's drought tolerance and adaptable cultivation requirements makes it a crucial contributor to food security (Howeler *et al.*, 2013). Furthermore, cassava is utilized in the production of various food items, including flour, which can replace wheat flour in baked goods, pasta, and bread, thereby providing a gluten-free option (Ceballos *et al.*, 2008). Cassava has also been employed in traditional herbal medicine due to its notable pharmacological properties, which include antioxidant, anticancer, antibacterial, and anti-inflammatory effects (Parmar *et al.*, 2017; Coêlho *et al.*, 2020). Beyond its medicinal uses, cassava serves as a crucial raw material in the production of bioethanol, starch, and animal feed, thereby contributing to rural development and energy generation (Torkpo & Amponsah, 2024; Rosário, 2023).

Despite its value as an energy source, cassava contains cyanogenic glycosides, and linamarin which can release toxic hydrogen cyanide upon hydrolysis, posing a risk to human health (Akinpelu *et al.*, 2011). To mitigate this risk, processing methods like fermentation are crucial in detoxifying cassava roots, significantly impacting its nutritional value and microbial load while reducing toxicity (Yadav *et al.*, 2021). Details on the effect of fermentation on nutritional flour of cassava are not well documented. Therefore, this study is designed to evaluate the effect of fermentation time on the nutritional profile and microbial load of cassava flour, thereby providing valuable insights into optimal processing practices that yield safer and nutritionally enhanced products.

Materials and Method

Study Area: The cassavas used in this research were purchased from Bokkos Main Market, in Bokkos Local Government Area of Plateau State, Nigeria and were transported to the National Veterinary Research Institute, Vom and processed within 24hours.

Cassava Flour Production: Cassava roots were peeled, washed, sliced, and fermented naturally in triplicates, in water for specified time frames of (0hr, 24hrs, 48hrs, and 72hrs), following the method of Lagnika *et al.* (2019). Post-fermentation roots were dried, milled, and sieved to obtain flour.

Proximate Analysis: Proximate analysis of the fermented roots was carried out to determine its nutritional compositions. Ash content was determined using the method of Pearson, crude fibre content was determined by the tricyclic acid TCA method (IITA, 2005), moisture content was determined according to the oven method of AOAC (AOAC, 2012), fat content was determined using the Soxhlet extraction, protein content was determined by the Micro-Kjeldahl method as described by (Lin, 2010).

Determination of Vitamin C Level: Vitamin C levels were determine from the sample at different fermentation times using the iodimetric method as described by Raman et al., (2022).The principle is based on the conversion of I_2 to iodide (I^-) in a reversible reaction. Here, I_2 is reduced to I^- and ascorbic acid is oxidized to dehydroascorbic acid. When the ascorbic acid is completely oxidized to dehydroascorbic acid, the excess iodine reacts with the starch indicator and forms a blue-black starch iodine complex. This is the endpoint of the redox titration.

Microbial Analysis

This was done following the procedure described by Obueh *et al.* (2014), the microbial load of fermented cassava roots was determined via the pour plate technique. 1 ml sample was serially diluted in sterile distilled water, and 0.1 ml aliquots were inoculated onto Nutrient Agar, MRS agar, Czapek Dox agar and then Sabaraud Dextrose Agar for fungal count. After counting the resulting colonies, representative strains were further sub-cultured and identified. Bacterial identification followed the criteria in Bergey's Manual of Determinative Bacteriology, while fungal isolates were analyzed using the wet mount method.

Microbial Load Assessment: Total bacterial count (TBC) and total fungal count (TFC) were performed at different fermentation intervals (0hr, 24hr, 48hr, 72hr) using steps outline by Obi *et al.* (2019).

Determination of Cyanide in Fresh Cassava Roots and Fermented Cassava Flour

The cyanide content were analyzed using the picrate method as described by Abdullahi *et al.*, (2014). Fresh cassava roots and fermented cassava flour from fermented roots were pulverized and analyzed for total cyanogens using picrate test method. The fresh cassava root samples were peeled and washed to remove debris. The roots were grated and 2g of the sample was weighed and dissolved in 20cm^3 of distilled water. The mixture was left for 24 hours for cyanide extraction. The mixture was filtered using filter paper and 80cm^3 of alkaline picrate solution was added to the filtrate and boiled in a test tube inside a water bath. Change in coloration from yellowish to reddish brown was observed indicating the presence of cyanide. The absorbance of the sample was read using spectrophotometer at a wavelength of 490nm. The cyanide concentrations were determined by interpolating its absorbance value on the cyanide concentration curve.

Results

The result from table 1 shows that the total bacterial count (TBC) in cassava flour was relatively low at 0 hours (3×10^4 CFU/g), but increased substantially upon fermentation, peaking at 48 hours (2.34×10^5 CFU/g). A notable increase in TBC was observed within the first 24 hours (1.6×10^5 CFU/g), indicating the establishment of an active microbial community dominated by fermentative organisms. The bacterial succession pattern during fermentation reveals a shift from a diverse microbial community consisting of Coagulase Negative Staphylococcus species (CoNS), *Bacillus spp*, and *Klebsiella spp*., to one dominated by lactic acid bacteria (LAB), particularly *Lactobacillus spp*. Table 2 shows the total fungal count (TFC) starting at 1.2×10^4 CFU/g with an increased slightly by 24 hours and more substantially by 48 and 72 hours. The fungal population dynamics during fermentation revealed that at first, both *Rhodotorula spp*. and *Candida spp*. were present at 0 and 24 hours. However, by 48 hours, *Candida spp.* became the sole fungal species.

The result in Table 3 revealed that moisture content decreased gradually with longer fermentation periods. The crude protein content exhibited a slight but consistent increase with fermentation, potentially due to microbial activity synthesizing amino acids or proteinaceous compounds. The crude fibre content remained relatively stable, with minimal changes indicating that the flour retains its value as a good source of dietary fibre. A slight increase in lipid content was observed while the ash content, reflecting total mineral content, remained stable throughout fermentation. The nitrogen-free extract, representing digestible carbohydrates, showed slight fluctuations but remained generally stable as the metabolizable energy values exhibited minor variations with no discernible trend, while the calcium and phosphorus content showed no clear pattern. Table 4 shows that pH reduced to acidic by 72 hours while the hydrogen cyanide levels also reduced with increased fermentation time.

Table 1: Total Bacterial Count (TBC) and succession from fermented cassava flour

Sample (Fermentation Time)	TBC (CFU/g)	Identified Bacteria
0 hour	3×10^4	CoNS, *Bacillus subtilis*, *Klebsiella aerogenes*
24 hours	1.6×10^5	CoNS, *B. subtilis*, *Lactobacillus* spp.
48 hours	2.34×10^5	*Lactobacillus* spp., CoNS, *B. subtilis*
72 hours	2.78×10^4	*Lactobacillus* spp.

Key: CoNS= Coagulase Negative Staphylococcus spp.

Table 2: Total Fungal Count (TFC) and isolates from fermented cassava flour

Sample (Fermentation Time)	TFC (CFU/g)	Fungal Isolates
0 hour	1.2×10^4	*Rhodotorula spp.*, *Candida spp.*
24 hours	1.4×10^4	*Rhodotorula spp.*, *Candida spp.*
48 hours	2.7×10^4	*Candida spp.*
72 hours	2.9×10^4	*Candida spp.*

Table 3: Proximate Analyses of fermented cassava flour

Time	Moisture (%)	Crude Protein (%)	Crude Fibre (%)	Lipids (%)	Ash (%)	NFE (%)	M.E. (kcal/100g)	Calcium (%)	Phosphorus (%)	Vitamin C mg/100g
0 hour	14.14	1.89	16.00	0.20	2.30	65.46	365.48	0.16	0.04	0.69
24 hours	13.12	1.92	16.03	0.21	2.31	66.41	361.76	0.15	0.03	0.35
48 hours	13.04	1.97	16.07	0.24	2.30	66.38	366.22	0.17	0.04	0.17
72 hours	12.88	1.99	16.07	0.25	2.29	66.52	364.34	0.15	0.03	0.12

NFE means Nitrogen Free Extract and M.E means Metabolizable Energy

Table 4: Cyanide and pH levels of cassava flour samples.

Sample (Fermentation Time)	pH	Cyanide level (mgHCN equivalents /10g sample)
0 hour	7.4	0.024
24 hours	4.9	0.023
48 hours	4.5	0.016
72 hours	4.3	0.004

Discussion

The total bacterial count (TBC) in cassava flour showed that the bacterial load was relatively low at 0 hours (3×10^4 CFU/g), but increased upon fermentation, peaking at 48 hours (2.34×10^5 CFU/g) suggesting that the microorganisms were able to utilize the fermentable substrates in the cassava mash effectively. Notable increase in TBC was observed within the first 24 hours (1.6×10^5 CFU/g), indicating the establishment of an active microbial community dominated by fermentative organisms. The peak at 48 hours aligns with the optimal growth period for many fermentative bacteria, particularly *Lactobacillus species*, which became dominant during this period. However, by 72 hours, the TBC declined to 2.78×10^4 CFU/g, potentially due to factors such as nutrient depletion, accumulation of inhibitory metabolites, or unfavourable environmental conditions resulting from the fermentation process as also reported by Obi *et al.*(2019) and Okoko. (2011).

The bacterial succession pattern during fermentation reveals a shift from a diverse microbial community that consisted of coagulase-negative *Staphylococci* (CoNS), *Bacillus subtilis*, and *Klebsiella aerogenes*, which are commonly found in soil and plant environments, to one dominated by lactic acid bacteria (LAB), particularly *Lactobacillus spp*. This transition is likely due to their ability to efficiently convert sugars into lactic acid, thereby lowering the pH as indicated by a drop in pH of the medium from 7.4 at 0hours to 4.3 by 72 hours. This can inhibit the growth of other microorganisms.(Adesulu *et al.*,2022) and (Colehour *et al.* 2014). By 48 hours, *Lactobacillus spp*. had become predominant, and was the sole identified genus by 72 hours. This dominance corresponds with the observed decrease in total bacterial count, suggesting that LAB inhibited competing microbes through acidification and possibly bacteriocin production (Pereira-Mathias *et al.* 2012; Yuliana *et al.* (2023). The total fungal count (TFC) exhibited a gradual increase throughout the fermentation period, contrasting with the bacterial population's decline at the later stage (Silveira & Furlong, 2007). Starting at 1.2×10^4

CFU/g, the fungal count increased slightly by 24 hours and more substantially by 48 and 72 hours. This progressive rise may indicate that certain fungi, such as acid-tolerant yeasts and molds, became more active as fermentation progresses (Aguilera, 2013). Monitoring fungal growth is crucial, as some species can be beneficial (e.g., yeast in fermentation), while others may pose spoilage or health risks if not controlled (Soares *et al.,* 2022). The findings suggest that fermentation time significantly impacts microbial load and composition in cassava flour, with 48-72 hours representing the peak period for bacterial activity and LAB dominance. However, the sustained presence of fungi at 72 hours, coupled with the decline in bacterial load, highlights the potential need for post-fermentation processing, such as drying or heat treatment, to ensure product safety (Chacha & Mamiro, 2019). The shift towards a Lactobacillus-dominated microbiota supports the safety and preservation benefits of fermentation, but careful consideration of fungal growth is necessary to optimise product quality.The fungal population dynamics during fermentation revealed a shift in composition over time. Initially, both *Rhodotorula spp*. and *Candida spp*. were present at 0 and 24 hours, indicating early fungal colonization likely originating from the cassava or environmental contamination. *Candida spp.*, an opportunistic fungus, thrives in moist, starchy environments and contributes to flavor and aroma development during fermentation. However, by 48 hours, *Candida spp.* became the sole fungal species, suggesting that the acidic environment and microbial competition during fermentation suppressed other fungi. *Candida's* resilience enabled it to survive longer fermentation periods, although its load may decrease over time. The decline in fungal diversity, particularly the disappearance of *Rhodotorula*, reflects the unfavorable condition created by fermentation, likely due to the drop in pH resulting from bacterial activity (Aboh *et al.,* 2021). Longer fermentation times (72 hours) may contribute to reduced fungal contamination, aligning with food safety goals (Ukom *et al.,* 2019). Although fungal isolates do not directly determine nutritional value, their activity during fermentation may influence enzyme release, facilitating partial breakdown of cassava starches and fibers, and potentially contributing to minor nutrient synthesis (Halake & Chinthapalli, 2020; Ayansina *et al.*, 2017).

The result in Table 3 reveals that moisture content decreased gradually with longer fermentation periods. This reduction is beneficial for flour production, as lower moisture levels improve shelf life and storage stability by discouraging microbial spoilage (Sağırlı *et al*., 2008). The crude protein content exhibited a slight but consistent increase with fermentation, potentially due to microbial activity synthesizing amino acids or proteinaceous compounds. The degradation of carbohydrates may also contribute to the apparent increase in protein concentration (Siburian *et al.*, 2019). The crude fibre content remained relatively stable, with minimal changes indicating that the flour retains its value as a good source of dietary fibre. A slight increase in lipid content was observed, possibly due to microbial lipolytic activity making lipids more extractable (Salami *et al*., 2017). The ash content, reflecting total mineral content, remained stable throughout fermentation, suggesting that minerals are neither lost nor concentrated significantly. The nitrogen-free extract, representing digestible carbohydrates, showed slight fluctuations but remained generally stable. The metabolizable energy values exhibited minor variations with no discernible trend, indicating that the energy value of cassava remains consistently high. The calcium and phosphorus content showed no clear pattern, suggesting that mineral content is maintained throughout fermentation. Overall, the results indicate that fermentation has a positive impact on the nutritional profile of cassava flour, with the 72-hour fermentation period appearing most favorable due to its high protein, fat, and low moisture content. The reduction in cyanide level from 0.024mg/10g to 0.004mg/10g indicates that fermentation contributes in reducing hydrogen cyanide level in fermented cassava flour, making it safer for consumption without effect on long term consumption.

Conclusion

This study demonstrates that fermentation is an effective method for reducing cyanide levels, improving the nutritional value and safety of cassava flour. However, the studies showed that the vitamin C level reduced with increased days of fermentation suggesting that consumers of fermented cassava flour should depend on other vitamin sources. A 72-hour fermentation period is optimal for achieving maximum nutritional benefits while minimizing residual toxins and microbial hazards. Adoption of a standardized 72-hour fermentation protocol for cassava flour production is recommended while further research on controlled fermentation using starter cultures to improve consistency and safety should be done.

Conflict of Interests

There is no conflict of interest

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