Fermentation Efficiency of Cassava using Indigenous Starter Cultures in Ebonyi State, Nigeria

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ABSTRACT

Cassava fermentation enhances detoxification, preservation, and food quality. This study assessed the microbial load, physicochemical characteristics and efficiency of cassava fermentation using autochthonous starter cultures isolated from Ikwo, Onicha and Okwor of Ebonyi state, Nigeria. A total of Fifteen samples of cassava effluent were aseptically collected and served as starter cultures. Microbial load was determined by the use of serial dilution followed by spread plate technique, while physicochemical parameters were analysed at intervals of 0hr, 12hrs and 24hrs of fermentation (odour, gas production, texture, and liquid separation) were monitored at 0, 12, and 24 hours of fermentation. Microbial counts ranged from 4.0×10^5 to 2.6×10^6 CFU/mL, with Okwor samples showing the highest average counts. Lactic acid bacteria were predominant across most samples, while some exhibited no detectable growth. A significant reduction in pH was observed from 6.03–6.53 at 0 hours to 4.97–5.97 at 24 hours, with samples 7 and 10 showing the most rapid acidification (pH 4.97 ± 0.03), indicating active fermentation. Samples 4 (Ikwo), 7, and 10 (Onicha) demonstrated the most efficient fermentation, characterised by rapid acidification and pronounced sensory changes. These results point to the possibility of using indigenous lactic acid bacteria (LAB) starter cultures to achieve better consistency in fermentation as well as enhanced safety of food and detoxification of cyanogenic glycosides. The study suggests the development and standardisation of indigenous starter cultures to improve consistency, safety, and scalability of cassava fermentation processes.

Keywords: *Cassava fermentation, lactic acid bacteria, indigenous starter cultures, physicochemical properties, and fermentation efficiency, food safety, cyanogenic glycosides detoxification*

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INTRODUCTION

Cassava (Manihot esculenta Crantz) is a staple crop extensively grown and eaten throughout sub-Saharan Africa and specifically important in Nigeria, providing food and a source of livelihood for the rural inhabitants (Borku, 2025). According to Ikuemonisan (2020), Nigeria is the largest producer of cassava, and carbohydrates are an essential component of a huge proportion of the population. Since cyanogenic glycosides can produce toxic hydrogen cyanide under poor conditions, efficient fermentation is necessary for the removal of toxin, the preservation of the foodstuff and enhancement of organoleptic characteristics (Adelusi *et al*., 2025).

Cassava fermentation is a spontaneous process where several microflorae, such as yeasts and lactic acid bacteria (LAB), are found to dominate depending on the initial microorganisms of the raw cassava root tuber, water and environment (Fawole and Kolapo, 2022). During the fermentation process microbial succession takes place as the microorganisms feed on the substrates, to produce metabolites that in turn result in a process that is de-toxic and results in enhanced palatability (Sheikh *et al*., 2024).

It is important to consider the quality of the microorganisms during the fermentation of cassava products. Diverse strains of yeasts and bacteria dominate the fermentation depending on methods and conditions adopted (De Guidi *et al*. 2023). Various genera were found to be involved in cassava fermentation where interaction exists between them that leads to some important properties such as acidity and flavour of the product (Halake and Chinthapalli, 2020).

Physicochemical properties like pH decrease during the fermentation process due to utilisation of sugars by microflora. Reduction of pH occurs as a result of the production of lactic acid by LAB, which helps in reducing the spoiling organisms, thus enhancing product quality and safety (Maleke *et al*., 2022). Factors influencing the fermentation efficiency include initial microbial load, substrate properties and conditions of fermentation (Sokra *et al*., 2026). Differences in conventional processing techniques between different villages affected the yield, the texture, microbial profile, quality and characteristics of the product. Recent studies also has shown the potential to control fermentation process with the use of lactic acid bacteria to reduce cyanide and enhance safety and quality of cassava based products.

Although the traditional cassava fermentation has been widely practised, the process is still empirical and uncontrolled and differs from one community to another. It consequently results in variation of fermentation efficiencies, product quality, and risk to health, arising from imperfect detoxification of the cyanogenic glucosides. In addition to this, There is a lack of comparative studies on performance between native starter cultures isolated from different communities with regard to their microbial populations dynamics, physicochemical parameters and fermentation effectiveness (Zhao *et al*., 2024). Thus, the study purpose was to determine the impact of starter cultures from the communities of Ikwo, Onicha, and Okwor of Ebonyi State, Nigeria, on microbial profile, physico-chemical attributes, and the fermentation performance of cassava. The fermentative characteristics of cassava and quality of products differ considerably among the diverse communities that ferment it because of the non-controlled fermentation process. This may lead to variability in microorganism profile, fermentation performance and the end products. However there is a limited information in literature on performance of diverse indigenous starters. The purpose of this study was therefore to find out the fermentation performance of starters from the Ikwo, Onicha and Okwor communities of Ebonyi State.

METHODOLOGY

Samples of cassava effluent were collected aseptically from fermentation sites of Ikwo, Onicha, and Okwor, three communities in Ebonyi State, in a sterile container and transferred under refrigeration to the laboratory. These communities (Ikwo, Onicha, and Okwor) were selected due to their established knowledge of conventional cassava processing and fermentation practices, which might affect the indigenous microbial populations. These effluents serve as indigenous starter cultures. They also consist of microbial consortia, mainly including lactobacillus and yeast, responsible for the spontaneous fermentation of cassava substrates. The cassava mash was prepared aseptically and inoculated using respective starter cultures. The samples were fermented at room temperature (28°C) and incubated for 24 hours. Samples were obtained from the fermentation mixture at times 0, 12, and 24 hours for estimation of microbial load. The 24 hours fermentation length was chosen due to preliminary investigations and reported findings that the initial stage of fermentation of cassava involves rapid activity of microbes, rapid decline in pH and onset of detoxification within this period. The serial dilution and spread plate methods were employed in the estimation of microbial load and reported as cfu/ml. Pure isolates were obtained by repeated subculturing and identified based on colony morphology, gram staining and biochemical tests like catalase, citrate, indole, urease and sugar fermentation. Physicochemical properties of the sample, such as pH, were determined by pH meter. Sensory characteristics (smell, gas evolution, texture, liquid segregation) and physical features were observed. Cross-inoculation studies were performed by using the starter cultures from different locations in the cassava

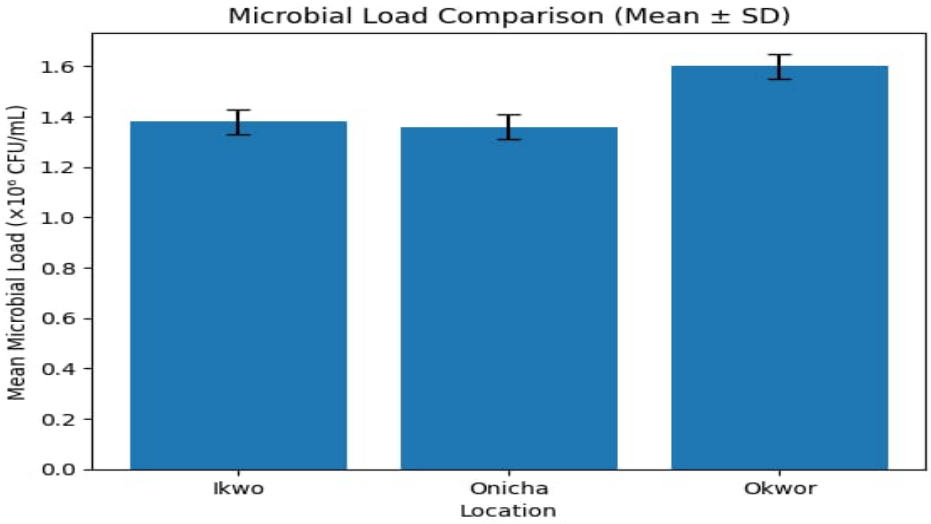

Figure 1: Microbial Load (CFU/mL) of the Cassava Effluent Samples

mash obtained from another location. The efficiency of the fermentation processes was examined by the speed of pH decrease and intensity of the sensory characteristics. All analyses were performed in triplicate, and the values obtained were analysed by one-way analysis of variance (ANOVA) at $p < 0.05$.

RESULTS AND DISCUSSION

The result of this research demonstrated the impact of indigenous starter cultures from Ikwo, Onicha and Okwor on the microbial count, physicochemical characteristics and fermentative efficiency of cassava. This study directly relates to the research objectives since there was a comparison between the distribution of the microbes, change in pH value and fermentation of the cultures selected from the three communities. The study also bridges a gap in previous literature byliterature by offering comparative data on indigenous starter cultures from various communities in Ebonyi State that have not been properly investigated as previously observed, as previous works centred more on controlled fermentation and single-strain sources.

Microbial Load

The microbial load of the cassava effluent samples ranges from 4.0 x 10 to 2.6 x 10 cfu/mL, with the samples from Okwor recording the highest average value, followed by Ikwo, and then Onicha. The variance was also statistically significant, $p < 0.05$. This shows that the environmental factors that vary according to geographic location are responsible for the variability of the microbial growth rates during fermentation processes. This result is in agreement with the report of Bamigbade et al., 2023, that fermenting cassava possesses a high population of microbes because readily fermentable substrates are present to support the high growth of microorganisms. Also, Egbune et al., (2023) reported that fermentations utilizing cassava as a base have dense microbial populations, especially during conventional production. Despite this, the number of microbes measured in this experiment is lower than that detected in several optimized fermentations in which microbial counts of >10 7 CFU/mL have been reported (Whang et al., 2024). This difference may have resulted from variation in fermentation control and the composition of substrates, as well as other variables in fermentation environment. Controlled fermentation systems can have near ideal conditions in the medium to maximize cell growth while conventional fermentation systems, such as this experiment, can have varying degrees of temperature, sanitation and microbial inoculum (Figure 1).

Microbial Distribution

Lactic acid bacteria dominated the most part of samples, indicating its major involvement in the fermentation of cassava (Table 1). Its dominance is in agreement with the results of Bamigbade et al. (2023) who reported that Lactic acid bacteria (LAB) have the capacity to thrive in the cassava fermentation conditions due to the acid tolerance and effective metabolism. This is in accordance with the finding of Atter et al. (2024) who reported that Lactic acid bacteria (LAB) are often dominant in spontaneous fermentation, owing to their competitive ability under acidic conditions and effective consumption of fermentable carbohydrates. Lactic acid bacteria metabolic process releases acids that inhibitions undesirable microbes. The non-LAB organisms were

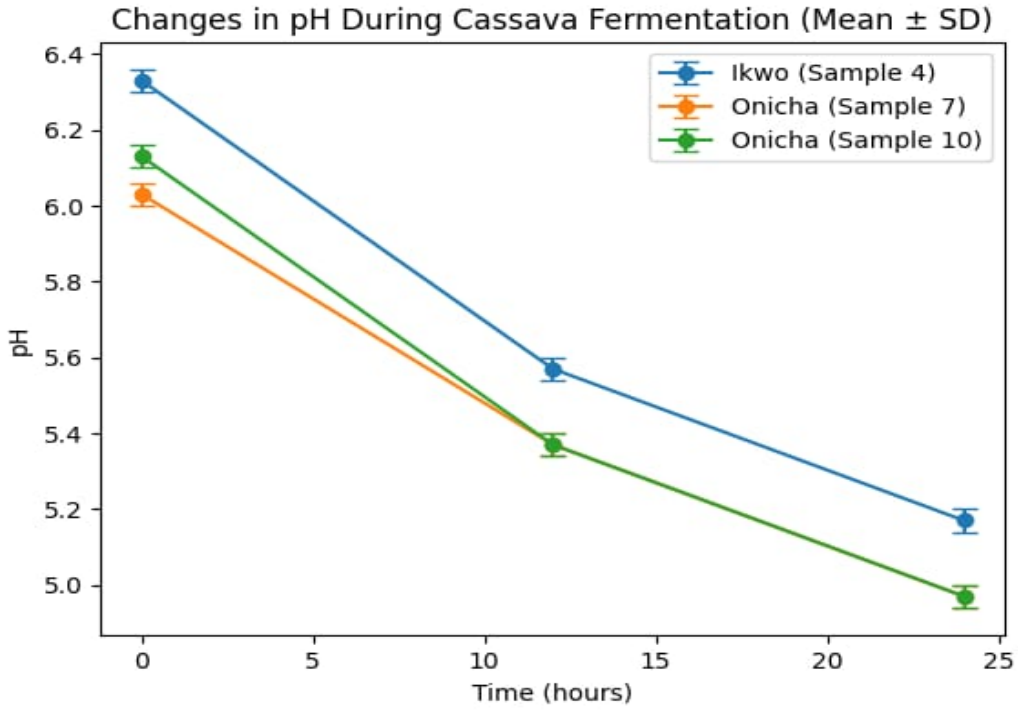

Figure 2: Changes in pH During Fermentation (0–24 Hours)

sometimes encountered which may have been introduced into the samples by contamination. Non-LAB organisms are a common characteristic in spontaneous fermentations due to absence of starter cultures. On the other hand, the absence of any microbial growth in the samples could be as a result of the presence of unfavorable environmental conditions (depletion of nutrients or accumulation of antimicrobial metabolites). This is supported by the reports of Ngounam et al. (2024) that metabolic by-products released by lactic acid bacteria inhibit less tolerant microorganisms.

pH Changes During Fermentation

A significant decrease ($p < 0.05$) in pH was observed across all samples, from an initial range of 6.03–6.53 at 0 hours to 4.97–5.97 at 24 h (Figure 2). Samples 7 and 10 exhibited the most rapid acidification, reaching a pH of 4.97 ± 0.03, indicating higher fermentation activity. This reduction in pH is attributable to the metabolic activity of LAB, which convert fermentable sugars into organic acids, primarily lactic acid. Similar findings were reported by Akter et al. (2025), who highlighted lactic acid production as the primary driver of acidification in fermentation systems. However, the final pH values recorded in this study are relatively higher than those reported in some controlled fermentations, where pH values below 4.5 are often achieved (Whang et al., 2024). This difference may be due to the shorter fermentation duration (24 hours) and the variability inherent in spontaneous fermentation systems. Furthermore, the variation in pH reduction among samples suggests differences in microbial activity and starter culture efficiency. Ndovie et al. (2025) emphasized that fermentation efficiency is strongly influenced by microbial composition and substrate-to-microorganism ratios, which may explain the observed differences among samples.

Sensory and Physical Changes

Fermented samples displayed significant differences in the degree of odour changes, gas production, texture changes, and liquid separation. Samples at 0 h remained as fresh cassava, while a slight sour odour and minimal gas production occurred by 12 h, demonstrating the beginning of fermentation, and 24 h showed a substantial difference from a sharp odour, high gas production, soft texture, and separated liquid (Table 2). These observations align with Atter et al. (2024), showing that LAB fermentation causes substantial sensory differences attributed to the formation of organic acid and volatile compounds, and gas formation is caused by the metabolic activity of the organism, primarily heterofermentative LAB and yeasts. Differences in the extent of sensory changes across the different samples were observed, with some samples displaying little changes (Table 3). This is different from observations

Table 2: Odour Changes During Fermentation.

S/N	Sample Code	0 h	12 h	24 h
1	1	Fresh cassava smell	Slight sour smell	Mild fermented aroma
2	2	Fresh cassava smell	Slight sour smell	Slight fermented odour
3	4	Fresh cassava smell	Sour smell	Strong fermented smell
4	5	Fresh cassava smell	Mild cassava smell	Weak fermentation smell
5	6	Fresh cassava smell	Slight sour smell	Mild sour aroma
6	7	Fresh cassava smell	Sour smell	Strong sour fermented smell
7	9	Fresh cassava smell	Slight sour smell	Moderate fermented smell
8	10	Fresh cassava smell	Sour smell	Strong sour smell
9	11	Fresh cassava smell	Slight sour smell	Moderate sour smell
10	15	Fresh cassava smell	Slight sour smell	Mild fermentation smell

Table 3: Texture Changes During Fermentation Using Starter Culture.

S/N	Sample Code	0 h	12 h	24 h
1	1	Hard	Slightly soft	Soft
2	2	Hard	Slightly soft	Soft
3	4	Hard	Soft	Very soft
4	5	Hard	Hard	Slightly soft
5	6	Hard	Slightly soft	Soft
6	7	Hard	Soft	Very soft
7	9	Hard	Slightly soft	Soft
8	10	Hard	Soft	Very soft
9	11	Hard	Slightly soft	Soft
10	15	Hard	Slightly soft	Moderately soft

Table 4: Gas Production during Fermentation Using Starter Culture**.**

S/N	Sample Code	0 h	12 h	24 h
1	1	No bubble	Few bubbles	Moderate bubbles
2	2	No bubble	Few bubbles	Little bubbles
3	4	No bubble	Moderate bubbles	High bubbles
4	5	No bubble	No bubble	No bubble
5	6	No bubble	Few bubbles	Moderate bubbles
6	7	No bubble	Moderate bubbles	High bubbles
7	9	No bubble	Few bubbles	Moderate bubbles
8	10	No bubble	Moderate bubbles	High bubbles
9	11	No bubble	Few bubbles	Moderate bubbles
10	15	No bubble	Few bubbles	Little bubbles

where uniform results were presented from fermentation under controlled conditions (Whang et al., 2024), and it could be attributed to differing indigenous microbial populations present in starter cultures and metabolic activity among them. Moreover, the subjective nature of observed sensory characteristics could influence comparability with work done with trained sensory panellists. More structured sensory evaluation might be recommended.

Fermentation Efficiency

The fermentation efficiency of the cassava was obtained in sample 4 (Ikwo), 7 and 10 (Onicha) that showed quick reduction in pH, significant changes in sensory attributes, significant increase in gas production and texture soften which are indication of intense microbial activity and proper breakdown of substrate (Table 4). This is in line with the observation made by Akter et al. (2025) that efficient fermentation processes characterized by fast rate of acidification and high level of LAB metabolism. Similar conclusion was drawn by Ngounam et al. (2024) when they asserted that the efficiency of fermentation process is dependent on the metabolic capability of the microbial populations. On the contrary, these diverse fermentative capabilities in various samples clearly indicate drawbacks associated with the conventional fermentation processes. Although efficient fermentation systems are capable of providing predictable outcomes (Whang, et al., 2024), the spontaneous fermentation used in this study does not provide such consistencies as a result of varying microbial populations and varying environment. This data provides insights into the food safety and food quality aspects because inconsistent fermentation may have implication in total degradation of cassava toxins (Tables 5-7).

Table 5: Liquid Separation during Fermentation using Starter Culture.

S/N	Sample Code	0 h	12 h	24 h
1	1	None	Small	Moderate
2	2	None	Small	Small
3	4	None	Moderate	High
4	5	None	None	Small
5	6	None	Small	Moderate
6	7	None	Moderate	High
7	9	None	Small	Moderate
8	10	None	Moderate	High
9	11	None	Small	Moderate
10	15	None	Small	Small

Table 6: Optimal Fermentation Performance at 24 Hours.

Sample Code	Location	Selected Replicate	0 h pH	24 h pH	Odour Intensity	Gas Production	Texture	Liquid Separation	Fermentation Level
4	Ikwo	**4**	6.3	5.2	Strong fermented	High	Very soft	High	Optimal
7	Onicha	**7**	6.0	5.0	Strong sour/ fermented	High	Very soft	High	Optimal
10	Onicha	**10b**	6.1	5.0	Strong sour/ fermented	High	Very soft	High	Optimal

Table 7: Comparison of Microbial Load, pH Variation, and Fermentation Efficiency in Cassava Fermentation Systems (Present Study vs. Recent Studies, 2023–2026).

Study	Microbial Count	pH Change	Fermentation Efficiency
Present Study	$4.0 \times 10^5 – 2.6 \times 10^6$ CFU/mL	6.5 → 4.97	High (high LAB domination, quick acidification and rapid sensory development)
Bamigbade et al. (2023)	~10^6 CFU/mL (LAB dominant)	6.2 → 4.2	High efficiency of cassava effluent system during fermentation
Egbune et al. (2023)	High microbial load in spontaneous cassava fermentation	~6.5 → 4.3	High activity during traditional cassava processing
Whang, et al. (2024)	~10^5–10^6 CFU/mL (optimized LAB medium)	6.8 → 4.5	High efficiency of optimal fermentation conditions
Atter et al. (2024)	LAB dominant population in spontaneous fermentation	~6.3 → 4.1	High as result of LAB tolerance to acid and utilization of substrates
Akter et al. (2025)	LAB-driven fermentation systems	~6.0 → 4.0	Very high in regulated lactic acid fermentation process system

Conclusion and Recommendations

This study demonstrated that indigenous starter cultures significantly influence the microbial activity, physicochemical changes, and overall fermentation efficiency of cassava. Lactic acid bacteria were predominant and played a central role in acidification, detoxification, and improvement of product quality. Rapid pH reduction and pronounced sensory changes observed in selected samples indicate enhanced fermentation performance. However, variability among samples highlights the inconsistency of traditional fermentation processes. Therefore, the development and standardization of effective starter cultures are essential for improving product safety, quality, and scalability. Future studies should focus on molecular identification of microbial communities and optimization of fermentation conditions to achieve controlled and reproducible outcomes.

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