

Fermentation of Cassava Leaves Using Microbial-Nutrient Additives: Impact on HCN and Fiber Content

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Abstract. This study aimed to evaluate the effectiveness of cassava leaf (*Manihot esculenta* sp.) fermentation using a combination of microbial (commercial EM4 and local microorganisms from fruit, MOL) and a nutrient additive (commercial Viterna) on the content of hydrocyanic acid (HCN), neutral detergent fiber (NDF), acid detergent fiber (ADF), and pH. The study used four treatments and five replications, namely: T0 (fresh cassava leaf without additives), T1 (fermentation with 40% EM4, 20% ml Viterna, and 40% ml MOL), T2 (20% EM4, 40% Viterna, and 40% MOL), and T3 (40% EM4, 40% Viterna, and 20% ml MOL). Each treatment was applied at a total rate of 0.5% (v/w) fresh cassava leaves. The samples were fermented anaerobically for 14 days. The data were analyzed using analysis of variance based on a completely randomized design and Duncan's further test. The results showed significant differences ($P < 0.05$) in HCN, NDF, ADF and pH levels among the treatments. The content of HCN T2 (2.79 ± 0.93 ppm) and T3 (3.43 ± 0.56 ppm) was lower than that T0 and T1. The lowest pH values were found in T2 (4.01 ± 0.00) and T3 (4.02 ± 0.02). The lowest NDF and ADF values were found in T3, with $42.55 \pm 0.23\%$ and $32.08 \pm 0.13\%$, respectively. It can be concluded that cassava leaf fermentation using a combination of microbial-nutrient additives in T3, effectively reduced the HCN content and improved the fiber quality.

Keywords: cassava leaves fermentation, microbial-nutrien, hydrogen cyanide (HCN), fiber quality

1 Introduction

Cassava leaves (*Manihot esculenta* sp.) are an agricultural byproduct with the potential to be used as animal feed. Cassava production produces abundant post-harvest byproducts, such as shoots, leaves, stems, and discarded tubers. Cassava shoots comprise 7% leaves and 12% twigs, whereas cassava stems comprise 89.1% of the total plant biomass. Cassava leaves contain approximately 28-32% protein, 12-16% carbohydrates, 1% lipids,

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minerals, calcium, and iron [1]. Although cassava leaves have a high nutritional value, the utilization of cassava leaves is limited by the presence of toxic natural antinutrient content. Antinutrients include cyanogenic glucosides, polyphenols, oxalates, tannins, phytic acid, and high fiber. Toxic compounds of cyanogenic glycosides, especially linamarin and lotaustralin in the leaves, are broken down by the enzyme linamarase to produce cyanohydrin which is further broken down by an enzyme called hydroxynitrile lyase to produce hydrogen cyanide (HCN) and toxic ketones [2]. Consuming large amounts of cyanogens can cause acute poisoning in livestock. Sheep are more susceptible to cyanide poisoning because microorganisms in the rumen can convert cyanogenic glycosides into cyanide [3]. Therefore, to utilize cassava leaves, detoxification processes such as fermentation or ensiling must reduce the cyanide content to a level safe for livestock consumption.

Fermentation is a processing method based on the principle of converting carbohydrates or organic acids into alcohol using microorganisms under certain conditions. During the fermentation process, the linamarase enzyme degrades cyanogenic glycosides into hydrogen cyanide (HCN), which then evaporates and is further degraded by fermentation microorganisms by breaking down toxic compounds, increasing the nutritional value [4]. Several studies have shown that fermented cassava leaves can reduce cyanide levels. Fermentation using *Lactobacillus* can reduce cyanide levels by more than 70% [5]. However, the use of a single fermentor still requires a longer time for detoxification and efficiency in breaking down fiber [4].

Along with the increasing need for efficient and locally based animal feed innovation, the use of a combination of inoculants and nutrient additives used by the community is a new approach that has not been widely explored [6]. The inoculants used included EM4 (*Lactobacillus* sp., *Saccharomyces* sp., *Actinomyces*), and MOL (local microorganisms resulting from the decomposition of organic waste). Meanwhile, the commercial nutrient additive for fermentation is Viterna, which contains nutrients as a source of protein, minerals, and energy to support microbial growth. The combination of these three is expected to produce a synergy of microbial activity that can break down the toxic compounds found in cassava leaves. Therefore, this study was conducted to find the most effective combination of inoculants and nutrient additives in reducing HCN levels and improving fiber in fermented cassava leaves.

2 Materials and Methods

This research was conducted in the Animal Husbandry Study Program, Faculty of Agriculture, Universitas Sumatera Utara. The study lasted for three months, from August 2025 to October 2025. This study did not involve human participants or live animals. All experimental procedures were conducted using plant-based materials and microbial fermentation techniques in accordance with standard laboratory safety.

2.1 Experimental procedure

The materials used in the study were fresh cassava leaves (*Manihot esculenta* sp.), EM4, Viterna and local microorganisms (MOL). EM4 is a commercial liquid probiotic that every liter contains *Lactobacillus* sp. (5×10^6 CFU/ml), *Saccharomyces* sp. (1.5×10^6 CFU/ml), *Rhodopseudomonas palustris* (1.0×10^6 CFU/ml). Viterna is a nutrient additive that every liter contains 10 g of fish meal, 2.5 g of monocalcium phosphate, 7.5 g of brown sugar, 5 g of urea, 4 g of sugar, 0.25 g of sprouts, 0.33 g of dolomite, 1.25 g of NaCl, 0.29 g of rice hulls, 0.14 g of honey, 0.21 g of coconut water. The local microorganisms (MOL) used in this study consisted of rice bran, molasses, and fruit waste. All materials were homogenized with a ratio of 1 : 1 : 3 (w/v/w) and stored under anaerobic conditions for 21 days. After the MOL was

ready for use, the cassava leaves and stalks were chopped using a chopper machine. Then, they were homogenized according to the following treatments : T0 (Fresh cassava leaf without additives), T1 (fermentation with 40% EM4, 20% ml Viterna, and 40% ml MOL), T2 (20% EM4, 40% Viterna, and 40% MOL), and T3 (40% EM4, 40% Viterna, and 20% ml MOL). Each treatment was repeated five times. Each treatment was applied at a total rate of 0.5% (v/w) fresh cassava leaves, equivalent to 5 ml per kg of material. The samples were fermented anaerobically for 14 days.

2.3 Data collection

2.3.1 pH

The pH of the fermented cassava leaves was measured using a calibrated HANNA pH meter. A 10 g sample was mixed with 100 ml of distilled water, then homogenized using a blender for 2 min and allowed to stand at room temperature for 30 min. The mixture was then filtered through Whatman No. 1 filter paper, and the pH of the filtrate was measured. Three measurements were recorded for each sample.

2.3.2 HCN analysis

A 10 - 20 g sample of finely ground sample was weighed and macerated with 100 ml of distilled water to a Kjeldahl flask for two hours. After maceration, an additional 100 ml of distilled water was added, and steam distillation was conducted. The distillate was collected in an Erlenmeyer flask containing 20 ml of 2.5% NaOH solution, and distillation was terminated when the total distillate volume reached 150 ml. Subsequently, 8 ml of NH₄OH solution and 5 ml of 5% KI were added, and the solution was titrated with 0.02 N AgNO₃ until appearance of turbidity.

2.3.3 Fiber analysis (NDF and ADF)

Fermented cassava leaves samples from each treatment were analyzed or neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents using the Van Soest detergent method.

2.4 Data analysis

The data were analyzed using analysis of variance (ANOVA) based on a Completely Randomized Design (CRD), followed by Duncan's multiple range test, using SPSS software version 22.0.

3 Results and Discussion

The effect of microbial nutrients on the content of pH, HCN, NDF and ADF contents of fermented cassava leaves are presented in Table 1.

Table 1. Contents pH, HCN, NDF and AD fermented cassava leaves

Variable	Treatment			
	T0	T1	T2	T3
pH	4.30±0.26 ^a	4.34±0.00 ^a	4.01±0.00 ^b	4.02±0.03 ^b
HCN (ppm)	7.45±0.24 ^a	4.87±0.44 ^b	2.79±0.93 ^c	3.43±0.56 ^c
NDF (%)	45.69±0.98 ^a	45.08±0.18 ^a	43.88±0.97 ^b	42.55±0.23 ^c
ADF (%)	35.44±0.21 ^a	35.35±0.54 ^a	35.23±0.26 ^a	32.08±0.13 ^b

Means with different superscript letters within the same column indicate significant differences ($p < 0.05$).

3.1 pH

pH plays an important role in the success of fermentation. The results showed a significant effect ($P < 0.05$) of microbial–nutrient treatments on pH. Treatments T2 and T3 had a lower pH than T0 and T1. This indicates that the fermentation process in T2 and T3 was more effective than T0 and T1. The decrease in pH is likely due to the synergistic effect between microbes and nutrient additives. *Lactic acid bacteria* (LAB) grow more effectively due to the presence of nutrient additives as a source of microbial energy, resulting in increased lactic acid production and lower pH values. The low pH aims to prevent harmful bacteria from growing. Harmful bacteria will be inhibited if the pH is below 4.20. In this study, treatments T2 and T3 had a pH below 4.20. Acidic conditions not only aim to inhibit spoilage microorganisms but also support the reduction of HCN. A lower pH can increase the rate of degradation of cyanogenic glycosides in cassava leaves [6].

The results of this study are in line with previous studies reporting that the use of microbes and nutrient additives significantly reduced the pH of cassava leaf fermentation from control conditions [6]. However, unlike previous studies that generally used a single nutrient source, such as molasses or a limited combination with lactic acid bacteria [7]. This study showed that the integration of commercial inoculants, local microorganisms and nutrient additives was able to produce stable fermentation conditions with a pH below the critical threshold of 4.20. This approach suggests that the combination of more diverse microbial sources with specific nutrient support not only lowers the pH, but also has the potential to increase fermentation efficiency through synergy of microbial activity.

3.2 HCN (Hydrocyanic acid)

The results showed that fermentation using microbial-nutrient additives had a significant effect ($P < 0.05$) on HCN content. Treatments T2 and T3 contained lower HCN level than T0 and T1. This effect is attributed to the combination of EM4, MOL, and Viterna in T2 and T3, which creates an optimal balance between fermentative microorganisms and nutrient availability. Thus, the microorganisms found in MOL and EM4 (*Lactobacillus* sp., *Saccharomyces* sp., and *Rhodospseudomonas palustris*) can grow more effectively and degrade cyanogenic compounds, resulting in reduce HCN levels. In contrast to previous studies, which used a single type of inoculant or nutrient source, this study demonstrates that microbial diversity supported by specific nutrients enhances the efficiency of cyanogenic compound degradation. This finding extends previous reports of HCN reduction through fermentation using *Lactobacillus* sp. and *Saccharomyces* sp. separately [8] and MOL with nutrient additives [9].

The HCN content of fresh cassava leaves before fermentation ranges from 1984,4 to 2,000 ppm on a fresh weight basis [10]. After fermentation, the degradation occurs because microorganisms break down cyanogenic glycosides. During the fermentation process,

microbes produce enzymes that can break down cyanogenic glycosides, such as linamarin, found in cassava leaves. These enzymes hydrolyze linamarin into glucose and acetone cyanohydrin, which then decompose into HCN and acetone. This process causes most of the HCN to evaporate, significantly reducing the HCN content [11]. The purpose of this fermentation is to reduce HCN levels below the safe threshold. The HCN levels in this study were still below the recommended limit. The safe threshold for HCN content is less than 10 ppm [12].

3.3 NDF

NDF content indicates the cellulose, hemicellulose, and lignin content of feed ingredients. High NDF are associated with lower feed digestibility. Based on the results of this study, fermentation using microbial-nutrients resulted in a significant difference ($P < 0.05$) in NDF content. The lowest NDF content was found in the T3 treatment. In contrast to previous research [6], which emphasized the role of a single microbial inoculant or limited nutrient source, the T3 treatment in this study showed that the use of microbial-nutrient additives was able to increase fiber efficiency during fermentation. This may be due to T3 containing higher nutrient additives compared to other treatments. Thus, cellulolytic microbes can grow better and produce cellulase/hemicellulase enzymes. The activity of these enzymes accelerates the degradation of cell wall components in cassava leaves. The results of this study are in line with research [13] which reported that the use of LAB and additional nutrients during cassava leaf fermentation can significantly reduce NDF content.

Although the NDF content of this study was still higher than the results of research [6], which reported the NDF content of fermented cassava leaves at 33-40%. The NDF value of the fermented results in all treatments in this study was still lower than that of fresh cassava leaves, which were reported to have an NDF content of 60.30% [13], This indicates that microbial-nutrient formulations based on local resources can reduce NDF content.

3.4 ADF

ADF is a component of NDF, consisting of cellulose and lignin fibers in feed ingredients. ADF is often used in feed ingredient evaluation to predict energy content and digestibility. High ADF content in feed generally indicates lower digestibility and energy availability for livestock. This is because cellulose and lignin are more resistant to degradation by digestive enzymes and microbial fermentation [14]. In this study, statistical results showed a significant effect ($P < 0.05$) on ADF content. T3 had the lowest ADF content compared to other treatments. ADF is part of NDF, so the decrease in ADF content is in line with the NDF observed in T3. This indicates that the T3 treatment can degrade fiber more efficiently. Unlike previous studies that generally relied on a single inoculant or the addition of specific enzymes, this study integrated commercial microbes, local microorganisms (MOL), and nutrient additives, thereby supporting the simultaneous production of cellulolytic and ligninolytic enzymes. This synergy plays a role in weakening the structure of cassava leaf cell walls, reducing lignin inhibition of enzyme activity, and enabling cellulase enzymes to work more effectively in breaking down cellulose [14]

The results of this study are in line with research [15] who reported that fermentation with *Saccharomyces cerevisiae* and nutrient additives (molasses and rumen fluid) can reduce ADF content. The ADF value in this study was lower than the ADF value in the previous study. (6) who reported that the combination of microbes with enzyme additives in fermented cassava leaves can reduce ADF from 30.48% to 21-22%. However, the ADF content in fermented cassava leaves in each treatment was still lower than that of fresh cassava leaves, (13) reported the ADF content of fresh cassava leaves was 39.85%.

4 Conclusion

Fermentation of cassava leaves using a combination of microbial and nutrient additives significantly affected hydrocyanic acid (HCN), fiber (NDF and ADF), and silage pH. The T3 treatment effectively reduced HCN content and improved fiber quality.

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