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# Sensory Characteristics and Lactic Acid Content of Cassava Leaf Fermentation Using Microbial-Nutrient

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ABSTRACT
Cassava leaves have a potential to be an alternative feed as it has high crude protein content, as well as essential vitamins and minerals. This research aims to find the effective combination dose of inoculant and nutrient additive in improving sensory characteristics and lactic acid in fermented cassava leaves (*Manihot esculenta*). This study used four treatments and five replications. 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). All additives were applied at a total dose of 0.5% (v/w) to the fresh weight of the material, and fermented anaerobically for 14 days. Data were analyzed using analysis of variance based on a Completely Randomized Design and Duncan's test. The results showed that T3 produced the highest lactic acid content (P <0.05) compared to other treatments. Treatments T2 and T3 produced a brownish-yellow silage color that was significantly different (P<0.05) from treatments T0 and T1, which showed a yellowish green color. The aroma produced in T2 and T3 was moresour (P<0.05) than T0. The treatment did not have a significant effect on silage texture (P>0.05). The combination of inoculants and nutrient additives had a significant effect (P<0.05) on the mold coverage score, with a tendency for a decrease in the mold coverage score from T1 to T3. It was concluded that The combination of inoculants and nutrient additives can improve the quality of cassava leaf fermentation. The best result is obtained in T3 which produces the highest lactic acid content and improved sensory characteristics.

**Keywords:** Cassava, Fermentation, Characteristics, Lactid Acid

## 1. Introduction

Cassava leaf production is quite significant, especially in tropical areas where cassava is widely cultivated [1]. One hectare of cassava land will produce a DM yield of 0.7 – 4.5 tons/ha of cassava leaves,depending on the cassava variety, cultivation practices, and harvest interval [2]. Cassava leaves are known for their high crude protein (CP) content, as well as essential vitamins and minerals. Therefore, cassava leaves have the potential to be an alternative feed for livestock [1]. Cassava leaves contain 20-32% CP. Based on reports [3], the amino acid content of cassava leaves is similar to that of soybeans, but differs in the low content of several amino acids, such as methionine, isoleucine, and lysine. However, the use of cassava leaves as animal feed has limitations because they contain toxic compounds, namely cyanogenic glycosides, specifically linamarin and lotaustralin, which can release hydrogen cyanide (HCN), which is toxic if consumed by livestock [4].

One method that has been recognized as effective for increasing the nutritional value of cassava leaves is fermentation. The fermentation process utilizes microbes to degrade toxic compounds into substances that can be consumed by livestock. Fermentation of cassava leaves using inoculants *Lactobacillus* sp. and *Saccharomyces* sp. can reduce cyanide levels by up to 97.17% [5]. In addition to playing a role in detoxification, the addition of microbial additives also affects the quality of fermentation, as indicated by the increase in lactic acid levels in cassava leaf silage [6]. Sensory quality improvements are also produced from the fermentation process. Riyanti [7] reported that cassava leaf silage with different molasses levels showed a relatively soft texture. In addition to improving texture, the fermentation process can also improve the preservation of taste [8].

At the community level, fermentation is generally carried out through the production of silage using commercial microbial inoculants EM4 (*Lactobacillus* sp., *Saccharomyces* sp., *Actinomycetes*), and MOL (local microorganisms resulting from the decomposition of organic waste). In addition to the addition of inoculants, nutrient additives such as Viterna are also used, which contain nutrients to support microbial growth. However, the dosage of this combination of inoculants and nutrient additives still varies among the community and has not been widely explored scientifically.

High variability in application dosages remains a primary challenge in achieving consistent, high-quality silage. To date, information regarding the synergistic relationship between various inoculant ratios and nutrient supplements remains limited. Therefore, this study was conducted to find the most effective dosage of the combination of inoculants and nutrient additives on the sensory characteristics and lactic acid content of fermented cassava leaves.

## 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

### 2.1 Experimental procedure

The materials used in this study were cassava leaves (*Manihot esculenta*) as the main ingredient for fermentation. The microbes used for fermentation come from commercial products (EM4) and local microorganisms (MOL). The microorganism content in EM 4 per liter includes: *Lactobacillus* sp. $5 \times 10^6$ CFU/ml, *Saccharomyces* sp. $1.5 \times 10^6$ CFU/ml, *Rhodopseudomonas palustris* $1.0 \times 10^6$. MOL is obtained from microorganisms derived from a mixture of fruit waste, molasses, and rice bran. While the nutritional additives used come from commercial products, namely Viterna, each 1 liter contains 10 g of Fish Meal, 2.5 g of Monocalciumphosphate, 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, 0.14 g of Honey, 0.21 g of Coconut Water, 0.29 g of Rice Hulls.

MOL is made by homogenizing mixture of rice bran, molasses, and fruit waste in a ratio of 1:1:3 (w/v/w) was then fermented under anaerobic conditions for 21 days. Next, the cassava leaves and stalks were chopped using a chopper machine and mixed homogeneously with additives according to the treatment, 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 repeated five times. All additives were applied at a total dose of 0.5% (v/w) of the fresh weight of the material, equivalent to 5 mL per kg of cassava leaves, and the samples were fermented anaerobically for 14 days in a plastic barrel (silo). The period was selected because, during fermentation, lactic acid production increases while pH decreases and becomes more stable, thereby suppressing undesirable microorganisms and resulting in good-quality silage [9].

## 2.2 Data collection

### *2.2.1 Sensory Characteristic*

Assessment of sensory characteristics quality, including aroma, texture, color, and the presence of mold, is carried out through sensory (organoleptic) testing using indicators that refer to [10] modified in Table 1.

Table 1. Cassava leaf silage assessment indicators

| Criteria | Score | Characteristics |
|---|---|---|
| | 1 | Dark chocolate |
| | 2 | Dark chocolate |
| | 3 | Brownish yellow |
| Color | 4 | Yellowish green |
| | 5 | Boiled green leaves |
| | 1 | Rotten |
| | 2 | Not fresh, there is a strange smell |
| Aroma | 3 | A bit sour |
| | 4 | The acid is quite sharp |
| | 5 | Stinging acid |
| | 1 | Solid (lumpy, slimy, watery) |
| | 2 | Slightly dense (a bit lumpy, with mucus) |
| Texture | 3 | Medium (not too dense/soft) |
| | 4 | Moderately soft (a little crumbly, barely slimy) |
| | 5 | Soft (not lumpy, not slimy, crumbly) |
| | 1 | Lots of mold, surface evenly covered |
| | 2 | Lots of mold, covering most of the surface |
| Mold | 3 | There is mold, covering a small part of the surface. |
| | 4 | A little bit of mold, only visible in a few spots |
| | 5 | No mold, clean surface |

### *2.2.2 Lactic Acid Content*

Determination of total acid is carried out using the AOAC method [11] by weighing 10 g of sample, then homogenized with 30 ml of distilled water. The homogenized sample was then filtered using ordinary filter paper to separate the solids from the filtrate. A total of 10 ml of filtrate was then diluted again with 40 ml of distilled water (1:5 dilution) in an Erlenmeyer flask to make the solution more dilute and transparent. To each dilution solution, 2–3 drops of phenolphthalein indicator were added, then the solution was titrated using 0.1 N NaOH solution until it reached the titration endpoint. The total acid value was then expressed as lactic acid equivalents and calculated using the equation:

$$C = V(ml) \times 0{,}09 \times 10 \times 5$$

V = volume of 0.1 N NaOH solution used in titration (mL), C = concentration of lactic acid (% w/w).

## 2.3 Data analysis

The data were analyzed using analysis of variance (ANOVA) based on a Completely Randomized Design (CRD) and Duncan's further test with the help of SPSS software version 22.0.

## 3. Results and Discussion

The effect of microbial-nutrients on the content of fermented cassava leaves is shown in Table 2.

Table 2. Lactic acid and physical properties of fermented cassava leaves

| | Treatment | | | |
|---|---|---|---|---|
| **Variable** | **T0** | **T1** | **T2** | **T3** |
| **Lactic acid (%)** | 2.25±0.52$^{a}$ | 2.37±0.39$^{a}$ | 3.15±0.52$^{b}$ | 4.05±0.52$^{c}$ |
| **Physical Properties** | | | | |
| Color | 3.83±1.20$^{a}$ | 3.21±1.23$^{ab}$ | 3.03±1.15$^{b}$ | 2.83±1.36$^{b}$ |
| Odor | 3.14±0.79$^{a}$ | 3.59±0.95$^{ab}$ | 3.79±0.98$^{b}$ | 3.76±1.24$^{b}$ |
| Texture | 3.86±1.06 | 3.69±0.97 | 3.93±0.99 | 3.59±1.15 |
| Mold | 4.34±0.77$^{ab}$ | 4.45±0.57$^{a}$ | 4.00±0.93$^{bc}$ | 3.86±0.83$^{c}$ |

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

### 3.1 Lactic Acid

Lactic acid production during fermentation is a key indicator of fermentation success. Lactic acid bacteria (LAB) metabolize the carbohydrates found in cassava leaves and convert them into lactic acid [12]. The results of this study indicate that there is a significant difference ($P<0.05$) between treatments in terms of lactic acid content. T3 has the highest lactic acid content compared to other treatments. This indicates that the type and percentage of inoculants and nutrient additives in T3 can create optimal fermentation conditions. The lower proportion of MOL in T3 is thought to reduce competition from non-LAB microbes that are heterofermentative or less efficient in producing lactic acid, so that fermentation takes place more directly and stably. Liu [13] reported that the combination of strong LAB inoculants with balanced nutrient availability was more effective in increasing lactic acid production compared to increasing sugar sources without controlling the microbial population, because dominant LAB were able to utilize nutrient sources or substrates efficiently so that they could suppress the activity of microorganisms that formed unwanted by-products.

The high lactic acid levels in T3 not only indicate an increase in lactic acid percentage but also improve the quality of fermented cassava leaves. Lactic acid plays a role in rapidly lowering the pH at the start of fermentation, thereby inhibiting the growth of pathogenic microbes that damage feed quality. Furthermore, under these conditions, ammonia-N levels also increase, thereby increasing the shelf life of silage [14]. The results of this study are in line with the findings [6], which reported that cassava leaf silage supplemented with microbial additives produced higher lactic acid levels than silage without additives. However, the lactic acid levels in that study were in the range of 3.01–3.46%, which was still lower than the lactic acid levels obtained in the T3 treatment in this study.

### 3.2 Sensory Characteristic

The sensory characteristics of fermented cassava leaves are influenced by the interaction between microbes and nutrients, resulting in differences in taste, aroma, and texture. The results of the sensory assessment in this study are presented in Figure 1.

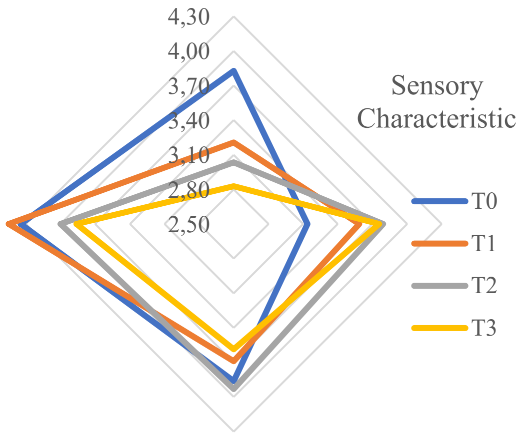


Figure1. Sensory characteristics of cassava leaf silage

**a. Color**

The initial color of fresh cassava leaves typically ranges from light green to dark green, with some varieties exhibiting reddish or purplish green hues on the veins [15]. Table 1 shows significant differences in the color of cassava leaf silage between treatments. Treatments T2 and T3 produced a brownish-yellow silage, in contrast to treatments T0 and T1, which showed a yellowish-green color. This color difference may be influenced by the intensity of fermentation and the amount of lactic acid during the silage process. Treatments T2 and T3 were known to have higher lactic acid levels than T0 and T1. This indicates a faster decrease in pH in T2 and T3, resulting in more intensive fermentation conditions. The increased acidity and anaerobic conditions trigger changes in leaf pigments through physiological biochemical reactions during fermentation [16]. Furthermore, during the ensilage process, chlorophyll is converted to pheophytin, causing the color to change from green to brownish-yellow. This color change is a common characteristic of silage with stable and effective fermentation and is often used as a general physical indicator of good-quality silage [17].

The results of this study are in line with Terefe's findings [5], who reported that cassava leaf silage supplemented with microbial nutrient additives tended to produce a yellowish-brown or greenish-brown color, indicating a successful fermentation process. Conversely, silage with suboptimal fermentation tended to show a dark brown to black color, indicating rotting or excessive heating due to inadequate silage conditions or ineffective microbial inoculation.

**b. Odor**

Fermentation significantly changes the aroma characteristics of cassava leaves through the metabolic activity of microorganisms that produce various volatile compounds. This process is crucial for enhancing the flavor of cassava leaves [5]. The results of this study showed a significant difference ($P<0.05$) in the aroma of fermented cassava leaves treated with microbial-nutrients. Treatments T2 and T3 showed higher values than T0 (control). The aroma produced in T2 and T3 was more acidic than that of T0. This is thought to be caused by the microorganisms involved in cassava leaf fermentation, especially lactic acid bacteria (LAB) and yeast, which play an important role in shaping the aroma profile [18]. *Lactobacillus plantarum* is known to be effective in lowering pH and increasing titratable acidity during cassava fermentation, which is important for flavor development and preservation [8]. In addition, the resulting aroma profile is also influenced by the microbial strains involved in fermentation, nutrient composition, fermentation media, and environmental factors such as temperature, pH, and fermentation duration [19].

The results of this study are in line with Sobriyan's findings [20], who reported that cassava leaf silage with added palm sugar and granulated sugar produced a sour aroma. This finding was also supported by Jayanti [21], who reported that the fermentation of green fodder with the addition of nutrients from sticky rice tape water produces a sour aroma character.

### c. Texture

Fermentation using microbial nutrients in cassava leaves did not have a significant effect (P>0.05) on the texture of cassava leaf silage. The resulting texture score ranged from 3-4, indicating a moderate to slightly soft texture without excessive mucus or clumping. The lack of differences in texture between treatments was due to the silage texture being largely influenced by the initial moisture content of the forage, fiber composition (NDF/ADF), and compaction during the silage process [22]. In this study, there was no difference in water content, fiber composition, and compaction level between treatments, so the physical condition of the resulting silage was relatively unchanged. This finding is supported by Zhang [23], who reported that natural fermentation of cassava leaves, even without the addition of microbial nutrients, still involves microbial enzymatic activity that degrades complex macromolecules. This process results in a reduction in hardness and an improvement in the material's physical characteristics, without producing significant differences in texture between treatments.

The results of this study are in line with Riyanti's finding [7], who reported that cassava leaf silage with different molasses levels exhibited a relatively soft texture. This finding is also supported by research by Herlina [24], who reported that cassava leaf silage produced a texture that was not wet, soft, and tender. Good silage has a recognizable physical structure, such as stems and leaves. If fermentation is less than optimal, it will result in structural damage, which ultimately affects the silage texture [25].

### d. Mold

The microbial-nutrient additive treatment had a significant effect (P<0.05) on the fungal score of cassava leaf silage. The score tended to decrease from T1 to T3. Treatment T3 showed the lowest fungal score and was significantly different compared to T0 and T1, but not significantly different from T2. The T3 value indicated the presence of fungi on a small portion of the surface, while in T0-T2, it was in the category of few fungi, only visible in a few spots. Although the highest percentage of lactic acid is in T3, which can generally inhibit the growth of destructive and pathogenic microorganisms, the presence of fungi is still possible in limited quantities. The survival of fungi can be influenced by various factors beyond lactic acid, such as certain fungal species that can tolerate acid [25].

The results of this study are in line with Jayanti's findings [21], who reported that fungus was still present in silage supplemented with sticky rice water. However, this finding does not align with Riyanti's [7], which stated that cassava leaf silage with different levels of molasses did not show the presence of fungi in the silage results.

## Conclusion

The combination of inoculants and nutrient additives can improve the quality of cassava leaf fermentation. The best result is obtained in 40% EM4, 40% Viterna, and 20% MOL, which produces the highest lactic acid content and improved sensory characteristics.

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