Fermentation profile and chemical composition of cassava root silage

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ABSTRACT

The use of cassava root silage for animal feeding is a suitable option for farmers who grow cassava as an alternative product and for cattle ranchers who have to deal with high prices of corn. Our objective was to determine the effects of cassava genotypes and the correction of soil acidity on the microbial population, fermentation characteristics, chemical composition, aerobic stability and losses of cassava root silage. We used a 2 × 3 factorial design in completely randomized blocks, with four replications. We evaluated two cassava genotypes (Caeté and Manteiguinha) and three methods of soil acidity correction (lime, gypsum, and lime+gypsum). The roots were harvested 11 months after planting, ensiled in PVC silos, and stored for 45 days. No interaction was observed between genotypes and soil acidity correction for any of the evaluated parameters. The silage of Caeté genotype showed the highest concentration of dry matter (421 g kg\(^{-1}\) fresh matter) and non-fibrous carbohydrates (893 g kg\(^{-1}\) dry matter), and the lowest concentrations of neutral detergent fiber (37.1 g kg\(^{-1}\) dry matter). No significant differences were observed among treatments for lactic acid bacteria, yeast and mold counts in silages. Both genotypes resulted in silages with an adequate fermentation profile and considerably high aerobic stability, but with high effluent loss. The Caeté genotype showed to be potentially better for silage production due to its higher dry matter recovery. Due to the high level of effluent loss, it is recommended to test the effect of a moisture-absorbing additive during the ensiling process of these cassava roots.

KEYWORDS: ensiling, feed conservation, fermentation, Manihot esculanta, sustainable crops

Efeito do genótipo e da correção de acidez do solo sobre o perfil fermentativo e a composição química da silagem de raiz de mandioca

RESUMO

O uso da silagem de raiz de mandioca na alimentação animal representa uma opção viável tanto para os produtores que cultivam mandioca (visando ter um produto para comercialização alternativa), quanto para os pecuaristas que compram milho a preços elevados. Assim, objetivou-se determinar o efeito de variedades e da correção da acidez do solo sobre as populações microbianas, características fermentativas, composição química, estabilidade aeróbica e perdas de silagens de raízes de mandioca.

O experimento foi conduzido em delineamento em blocos casualizados, em arranjo fatorial 2 × 3 com quatro repetições. Foram avaliados dois genótipos de mandioca (Caeté e Manteiguinha) e três tipos de correção de acidez do solo (calagem, gessagem e calagem+gessagem). Após 11 meses, as raízes foram colhidas, trituradas e ensiladas em silos do tipo PVC, onde permaneceram por 45 dias. Não observamos efeito de interação entre genótipos e correção do solo para os parâmetros testados. As silagens do genótipo Caeté apresentaram as maiores concentrações de matéria seca (421 g kg\(^{-1}\) matéria fresca) e carboidratos não fibrosos (893 g kg\(^{-1}\) matéria seca), e menores de fibra em detergente neutro (37.1 g kg\(^{-1}\) matéria seca). Não houve diferenças significativas entre tratamentos para bactérias ácido láticas, leveduras e mofos nas silagens. Ambos os genótipos resultaram em silagens com perfil de fermentação adequado e estabilidade aeróbica consideravelmente elevada, mas com elevadas perdas de efluentes. O genótipo Caeté apresentou condições potencialmente melhores para produção de silagem devido a sua maior recuperação de matéria seca. Devido à alta perda de efluente, recomendamos testar o efeito da adição de um aditivo absorvente de umidade durante o processo de ensilagem dessas raízes de mandioca.

PALAVRAS-CHAVE: conservação de alimentos, culturas sustentáveis, ensilagem, fermentação, Manihot esculenta

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INTRODUCTION

Cassava (Manihot esculenta Crantz) is a largely used crop in tropical regions of the world and Brazil is the fourth largest producer of cassava in the world (FAO 2020), with a total production of approximately 19 millions tons in 2020 (IBGE 2021). Almost 40% of the cassava production in Brazil comes from the northern Amazonian region, and Pará state is the largest producer in the country (IBGE 2021).

Cassava is used for human and animal feeding and is processed for several industrial purposes. In the Amazon region, cassava is a staple food for indigenous and non-indigenous populations, and is the main income source of smallholder farmers, which leads to the preservation of a range of genotypes cultivated in the region (Elias et al. 2004). The majority of cassava roots produced in the Amazon region is used to produce cassava flour, and also to produce starch, ethanol, alcoholic beverages and animal feed (Ajbola et al. 2012; Supapong et al. 2019; Coelho et al. 2020).

The diversification of uses of cassava roots is an important strategy to assure the maintenance and income of smallholder farmers (Santos and Santana 2012), because the market value of cassava flour is considerably variable (CEPEA 2020). In this context, the use of cassava roots as a substitute for corn in animal feeding is an interesting strategy, as corn is an expensive component of feed (Sudarman et al. 2016; Saree et al. 2017). The ensiling of cassava roots is an option to preserve this product through a fermentation process and to use it in diets for both ruminant and non-ruminant animals, such as cattle and swine, respectively (Loc et al. 1997; Vieira et al. 2017).

Cassava root silage preserves the nutritive value of the fresh product and decreases its concentration of hydrocyanidric acid, which is present in significant amounts in the roots (Loc et al. 1997; Chisté et al. 2010). Despite its high potential, there are few studies on cassava root silage, specially in Brazil (Loc et al. 1997; Silva et al. 2010; Vieira et al. 2017). The existing studies have not evaluated the characteristics of cassava root silage, which is the first step to evaluate the effectiveness of the ensiling process and the preservation of the nutritive value of the product. In addition, the fermentation process can be affected by genetic characteristics of ensiled cassava, fertilization and management of the crop. Soil acidity can also affect silage fermentation, as Amazonian soils have low natural fertility due to the presence of acids, aluminum toxicity and low base saturation (Soares et al. 2005). Soil-acidity correction measures such as liming are able to promote a better extraction of nutrients from the soil (Raij 1991), which can translate into larger substrates in the plant that will be available in the fermentation process.

Thus, our objective was to evaluate the fermentation characteristics and the chemical composition of cassava root silages produced with two genotypes cultivated with different soil acidity correctives.

MATERIAL AND METHODS

Study site

The experiment was conducted at Universidade Federal Rural da Amazônia – UFRA, in Belém, Pará state, Brazil (1°27’07”S, 48°26’13”W), at an altitude of approximately 11 m. The climate in the region is classified as Af (tropical humid) according to the Köppen classification system (Köppen 1918), is characterized by abundant rainfall throughout the year, a main rainy season between December and May, and a low rainy season from June to November, with average annual temperature of 26°C, and average annual precipitation of 2,774.3 mm (Alvares et al. 2014). The soil of the experimental area was classified as a dystrophic Yellow Latosol (Oxisol). (Santos, et al.; 2013). Five soil samples were randomly taken from a 0-20-cm depth within the experimental blocks, from which a composite sample was formed and analyzed for chemical properties in the Soil Chemistry Laboratory of UFRA (Table 1).

Procedures and experimental design

The experimental design was in completely randomized blocks, in a 2 x 3 factorial design with four replications (total of 24 experimental units). The studied factors were two cassava genotypes (Caeté and Manteiguinha) and three methods of soil-acidity correction [lime (L), gypsum (G), and L+G].

Based on the characteristics of the soil (Table 1), the amount of lime applied was 1.22 t ha⁻¹ (PRNT = 100%), using the exchangeable aluminum neutralization method (Cravo et al. 2016). Agricultural gypsum was applied at a dose of 1.2 t ha⁻¹, following the recommendation for annual crops for yellow oxisoil type by Sousa et al. (2007). The same amounts were used in the combined lime-gypsum treatment. Soil-acidity correctors were applied 20 days before planting the cassava stems (seeds). Fertilization followed the recommendation

| Table 1. Chemical composition of Yellow Latosol (Oxisol) samples collected at the campus of Universidade Federal Rural da Amazônia, Belém, Pará state, Brazil before the application of acidity correctives. |
|---|---|---|---|---|---|---|---|---|
| Depth (cm) | pH (H₂O) | N (%) | P (mg dm⁻³) | K (mg dm⁻³) | Na (mg dm⁻³) | Ca (cmol dm⁻³) | Mg (cmol dm⁻³) | Al (cmol dm⁻³) |
| 0-20 | 4.6 | 0.05 | 6 | 22 | 14 | 0.3 | 0.2 | 1.4 |

N = nitrogen; P = phosphorus (Mehlich extraction); K⁺ = potassium (Mehlich extraction); Na⁺ = sodium (Mehlich extraction); Ca²⁺ = calcium (KCl 1.00 mol L⁻¹ extraction); Mg²⁺ = manganesium (KCl 1.00 mol L⁻¹ extraction); Al³⁺ = aluminium (calcium acetate 0.05 mol L⁻¹ extraction).
for the state of Pará by Cravo et al. (2010). As nitrogen, phosphorus, potassium and micronutrient sources we used urea, triple superphosphate (TSP), potassium chloride (KCL), and FTE-BR12, respectively.

The cassava stems were cultivated with 1 m between rows and 0.5 m between plants, with five plants per plot and four replicates (plots) per treatment. Due to logistics, the cassava was harvested (and the material ensiled) over four weeks. Each week a plot of each treatment was harvested, and so the week of harvest and ensiling was used as the blocking criterion. Eleven months after planting, cassava roots were harvested. The blocking dates were 19 Jan, 20 Jan, 27 Jan and 30 Jan 2019. Each block contained six plots, one of each treatment. The roots were separated from the foliage and chopped in a stationary forage chopper (TRF 400, Trapp, Brazil) regulated to obtain a particle of approximately 2 cm. A sample of 500 g of the chopped cassava from each plot was separated for chemical characterization. Approximately 2.85 kg of chopped roots were placed in cylindrical PVC silos (volume = 0.0024 m\(^3\); packing density = 1.186 ± 131 kg m\(^{-3}\) fresh basis), containing 1.5 kg of dry sand covered with cotton cloth to absorb the effluent produced for later calculation of effluent loss. One silo was produced for each plot, totalling four silos per treatment. Each silo (experimental unit) was sealed, weighed and stored at the Animal Nutrition Laboratory of UFRA at room temperature (28.1 ± 0.9 °C) for 45 days.

At the end of the storage period, the silos were again weighed to quantify the dry matter recovery (DMR). The silos were opened, and the deteriorated portion of the silage (with visible mold) was removed before sampling. The empty silos and the sand were weighed for the calculation of the effluent loss (EL). The DMR and EL were calculated using the equations proposed by Jobim et al. (2007). We collected a sample of 500 g of the silage from each experimental unit for laboratory analyses.

The fresh silage samples were transferred to 5-L plastic buckets, which were stored at constant temperature of 25 ± 0.6 °C for seven days (168 hours). During this period, ambient temperature and silage temperature was recorded every four hours, the latter with a thermometer (Tpt101, Matsuri, China) inserted into the geometric center of the silage mass. Aerobic stability (AS) was defined as the number of hours the temperature of the silage remained stable before rising more than 2 °C above the ambient temperature (O’Kiely 1993). We also determined the time to reach the maximum temperature (HMaxT), maximum temperature (MaxT), minimum temperature (MinT), and temperature amplitude (AMP) (the difference between the maximum and minimum temperatures) of the fermentation process.

**Microbial population and fermentation profile**

The evaluation of microbial populations in the silage was carried out at the Animal Nutrition Laboratory of UFRA. For each replicate, an aqueous extract with 25 g of silage and 225 mL of sterile peptone water was prepared, which was homogenized manually for 3 min. Serial dilutions from 10\(^{3}\) to 10\(^{8}\) were prepared and plated on Petri dishes using the pour plate technique with Potato Dextrose Agar culture medium (Sigma-Aldrich Brasil Ltda) for yeasts and molds, and MRSA GAR (Sigma-Aldrich Brasil Ltda) for lactic acid bacteria (LAB). Plates were incubated in a germination chamber (BIOFOCO-BF2 CGFP 275) at 26 °C for three and five days for yeasts and molds, respectively, and the colony forming units (CFU) of each microorganism were identified based on their macromorphological characteristics and counted (Knop 2011). The plates for LAB were incubated at 35 °C for three days. After microbial analysis, we measured the pH of the aqueous extract using an electrode (Tekna-T-1000).

**Chemical composition**

The determination of the chemical composition of the harvested chopped roots (Table 2) and the silage were carried out at the Animal Nutrition Laboratory of UFRA. The samples were weighed and dried in a forced air oven (55 °C for 72 h) and then ground in a Willey knife mill (STAR-FT-80/2) with a screen size of 1 mm. The analysis of the concentration of dry matter (DM) (method 934.01), organic matter (OM) (method 923.03), and crude protein (CP) (method 978.04) followed the official methods of AOAC (1990).

**Table 2.** Chemical composition of the roots of two cassava (Manihot esculenta) genotypes planted in experimental plots at the campus of Universidade Federal Rural da Amazônia, Belém (Pará state, Brazil) using three types of soil acidity correction. Values are the mean of four in natura samples (before ensiling).

| Parameter                        | Genotype       | Caeté | Manteguinha |
|----------------------------------|----------------|-------|-------------|
|                                  | L              | G     | L+G         |
| Dry matter (g kg\(^{-1}\) FM)    | 412.0          | 398.0 | 399.0       |
| Organic matter (g kg\(^{-1}\) DM) | 959.0          | 950.1 | 939.9       |
| Crude protein (g kg\(^{-1}\) DM) | 24.7           | 24.0  | 22.6        |
| Ether extract (g kg\(^{-1}\) DM) | 2.9            | 2.8   | 2.4         |
| Neutral detergent fiber (g kg\(^{-1}\) DM) | 41.6           | 53.3  | 55.4        |
| Non fibrous carbohydrates (g kg\(^{-1}\) DM) | 889.8          | 869.7 | 857.4       |

FM = fresh matter; DM = dry matter; L = lime; G = gypsum; L+G = lime+gypsum; SEM = standard error of the mean.
The concentration of ether extract (EE) was determined in a fat extractor (Model: XT10- Ankom®; Method: Am-5-04) according to AOCS (2009). The concentration of neutral detergent fiber (NDF) was determined in accordance with method 973.18 of AOAC (1990) with the use of alpha amylase and without sodium sulfide. The concentration of non-fibrous carbohydrates (NFC) was calculated according to Detmann and Valadares Filho (2010), and ammonia nitrogen (NH₃-N) according method 941.04 of AOAC (1990).

**Statistical analyses**

All variables submitted to statistical tests were analyzed through the Shapiro-Wilk test for checking normality and through the Levene test for homogeneity of variance. Data from microbial populations were transformed to log₁₀ for normalization. All variables were submitted to analysis of variance, using the MIXED procedure in the SAS statistical package (SAS 2009). The statistical model used was:

\[ Y_{ijk} = \mu + b_i + a_j + \beta_k + (\alpha \beta)_{jk} + \epsilon_{ijk} \]

where:
- \( Y_{ijk} \) = experimental unit value in \( i \) block submitted to \( j \) variety and \( k \) soil correction;
- \( \mu \) = overall average;
- \( b_i \) = random effect associated with \( i \) block, \( i = 1, \ldots, 4 \);
- \( a_j \) = associated fixed effect with \( j \) variety, \( j = 1 \) e 2;
- \( \beta_k \) = fixed effect of \( k \) soil correction, \( k = 1, 2, 3 \);
- \( \alpha \beta_{jk} \) = fixed effect of variation between \( j \) variety with \( k \) soil correction;
- \( \epsilon_{ijk} \) = random error associated with the experimental unit in \( i \) block submitted a \( j \) variety and \( k \) soil correction.

**RESULTS**

There was no significant interaction between the factors for any of the evaluated variables. There was also no significant effect of acidity correction or genotype on microbiota variables (LAB, yeasts and molds counts) (Table 3).

Silages from cassava cultivated with gypsum and lime+gypsum had significantly higher HMaxT compared to lime only (Table 3). The Caeté genotype showed significantly higher values of pH, DMR, MaxT and AMP than the Manteiguinha genotype (P < 0.05 for all variables). Manteiguinha, in turn, showed significantly higher AS than Caeté (P < 0.01).

There was a significant effect of the correction of soil acidity on the concentration of EE and N-NH₃ (P < 0.01; Table 3), while the other parameters of chemical composition did not vary significantly among treatments (P > 0.05). Silages of the Caeté genotype showed significantly higher concentrations of DM and NFC, and lower concentration of NDF (P < 0.01) compared to Manteiguinha. The concentrations of OM and CP were not affected by genotype.

**DISCUSSION**

The use of lime and gypsum alone or in combination usually increases growth and root production in cassava plants because of the soil acidity correction in the superficial soil layer (0-20 cm) by lime and the positive effects of neutralizing toxic Al³⁺ and providing sulfur (S) and calcium (Ca) by gypsum.
The pH values observed in our fermentation profiles were within the range of 3.8 to 4.2 observed for well fermented silages (McDonald et al. 1991; Kung Jr. et al. 2018), indicating that the fermentation profile of cassava roots in our study can be considered adequate. The difference observed between the two genotypes was statistically significant but falls within the range of values considered normal in the fermentation profile. In addition, the final pH of the silage (after at least 30 days) by itself does not provide enough information about the fermentation process, which should be evaluated by the pH of the first days of fermentation, when undesirable microorganisms are inhibited (Silva et al. 2018). Based on that, the silage that shows the fastest pH drop during the beginning of the fermentation will probably show higher DM recovery. This could explain the higher DM recovery observed for the Caeté genotype. The lower DM recovery observed for the Manteiguinha genotype may be related to different fermentation pathways, that produce acetic acid and ethanol, that increase CO₂ production (Rooke and Hatfield, 2003).

Our effluent losses were high independently of the treatment, which was not expected for materials ensiled with 40% DM, as the cassava roots. However, these assumptions are based on the aerial parts of the plant, which have a high proportion of cell wall components (cellulose, hemicellulose and lignin) (Célino et al. 2014; Sorieul et al. 2016). After chopping, cassava roots form a dense mass from which a white liquid drips, which is reflected in the low concentration of NDF. It is known that the fiber has hygroscopic properties (Célino et al. 2014) and its low concentration could justify the high effluent production even in a material ensiled with 40% DM.

The aerobic stability of our silages can be considered high in comparison with corn and sorghum silages. Corn silages are more prone to aerobic deterioration (Ranjit and Kung 2000), mainly due to the high production of lactic acid produced during fermentation, which is used as substrate by yeasts (Pahlow et al. 2003). Lactic acid, acetic acid and soluble sugars

| Parameter | Soil acidity correction | Genotype | SEM | P-value |
|-----------|------------------------|----------|-----|---------|
|           | L          | G          | L+G | C      | M    | SC| GE| SC × GE |
| Dry matter (g kg FM⁻¹) | 399.7 | 397.9 | 398.1 | 421.0a | 376.1b | 11.25 | 0.98 | <0.01 | 0.61 |
| Organic matter (g kg DM⁻¹) | 957.8 | 953.1 | 953.1 | 956.7 | 952.6 | 2.44 | 0.40 | 0.24 | 0.52 |
| Crude protein (g kg DM⁻¹) | 22.1 | 22.1 | 21.8 | 21.6 | 22.3 | 0.24 | 0.95 | 0.46 | 0.63 |
| Ether extract (g kg DM⁻¹) | 3.7a | 2.6b | 3.5ab | 3.4 | 3.1 | 0.02 | <0.01 | 0.36 | 0.63 |
| Neutral detergent fiber (g kg DM⁻¹) | 41.9 | 44.8 | 44.8 | 37.1b | 50.6a | 3.63 | 0.64 | <0.01 | 0.28 |
| Non fibrous carbohydrates (g kg DM⁻¹) | 890.2 | 878.8 | 877.0 | 893.0a | 871.0b | 8.03 | 0.21 | <0.01 | 0.44 |
| Ammonia nitrogen (g kg TN⁻¹) | 56.1b | 59.7ab | 67.0a | 60.1 | 61.7 | 4.55 | 0.01 | 0.54 | 0.29 |

FM = fresh matter; DM = dry matter; TN = total nitrogen; L = lime; G = gypsum; L+G = lime+gypsum; C = Caeté; M = Manteiguinha; SEM = standard error of the mean; SC = soil acidity correction; GE = genotype; SC × GE = interaction between soil acidity correction and genotype. Means within group followed by different letters for a variable differ significantly by the Tukey test (P < 0.05).

(Zambrosi 2007; Vitti and Priori 2009). The correction of soil acidity with lime has been related to higher root production in cassava (Martins et al. 2018), while the application of gypsum promoted the increase in the dry matter of leaves in cassava genotypes, and the combination of lime and gypsum increased the root harvest rate (Nascimento et al. 2019). Such effects on productivity were not reflected in the characteristics of the silage in our study, including LAB, yeast and mold counts, as the fermentation process occurred within the expected for an adequate fermentation independently of the method of soil-acidity correction used. The values of LAB counts were higher than the average for tropical grass silage (4.73 cfu g⁻¹ of fresh matter; Gandra et al. 2017) and for corn silage (4.59 cfu g⁻¹; Oliveira et al. 2017).

**Table 4.** Chemical composition of cassava (*Manihot esculenta*) root silage of two genotypes planted using three types of soil-acidity correction at the campus of Universidade Federal Rural da Amazônia, Belém (Pará state, Brazil). Values are the mean of eight (acidity correction) or 12 (genotype) samples.

It is known that the activity of microorganisms influences the fermentation process during ensiling (Borreani et al. 2018). Although we did not analyze the microbiota during the early periods of the fermentation process, our silage-yeasts counts and DM-recovery values indicate that the yeast-growth rate was not high during the fermentation process, as our values for yeast count (3.0 to 3.3 cfu g⁻¹ of fresh matter) are within the adequate range proposed by Pahlow et al. (2003) and Kung et al. (2018). According to these authors, yeast counts above 5 log cf g⁻¹ indicate a high growth rate during fermentation that can affect the fermentation process by increasing DM losses, as observed in sugarcane silage (Ávila et al. 2010). The yeasts population in the silage is negatively correlated with aerobic stability (Schmidt and Kung Jr. 2010; Persson 2015) as they are usually the microorganisms that initiate the aerobic deterioration (Pahlow et al. 2003). The reason for this is that many yeasts are able to grow at pH 3.5, well below the pH of most silages (Muck et al. 2010). The yeasts population at the opening of the silos did not seem to affect the aerobic stability of the cassava silages, since the values remained below 5 log10 cfu g⁻¹. Silages that are actively deteriorating generally have values between 5.5 to 6.0 log10 cfu g⁻¹ (Santos et al. 2014).
are main sources of energy for microorganisms involved in the deterioration of silage (Mcdonald et al. 1991). Therefore, silages with a low content of these components may show high aerobic stability. The adequate fermentation profile and high aerobic stability of our silage may also indicate that other chemical compounds or metabolites in the silage are affecting microbial populations and preserving the silage.

The high aerobic stability observed in our silages can be explained by their high moisture content, which can result in an acetic fermentation of the silage (Kung et al. 2018). High humidity silages (> 70%) can be characterized by fermentation by heterolactic acid bacteria, enterobacteria, or even clostridia, which are microorganisms that produce acetic acid through their metabolism. (Mcdonald et al. 1991). This could explain the higher aerobic stability of the silage from the genotype with lower DMR, as a higher production of acetic acid would increase DM loss (Mcdonald et al. 1991). Aerobic stability could also result from yeast control by other compounds in the cassava, such as hydrocyanidric acid or tannins, by inhibiting spoilage microorganisms such as yeasts (Nasir and Hamad 2014).

**CONCLUSIONS**

Cassava root silage from two genotypes (Caeté and Manteigunha) cultivated with three types of soil-acidity correction showed an adequate fermentation profile, considerably high aerobic stability, and high effluent loss. The Caeté genotype had higher dry matter recovery, showing higher potential for silage production. Soil acidity correction with lime, gypsum or both in combination did not influence the characteristics of the silages in a meaningful way. Based on our results, the use of additives to improve the fermentation or aerobic stability is not needed for cassava root ensiling of these genotypes. However, due to the high production of effluents, the addition of a moisture-absorbent additive or the production of a total mixed ration during the ensiling process of cassava roots is likely necessary and should be formally tested.

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