Fermentation profile, aerobic stability, chemical and mineral composition of silages of mango combined with cocoa pod husk meal

Perfil fermentativo, estabilidade aeróbia, composição química e mineral de silagens de manga combinados com farelo de casca de cacau

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ABSTRACT
The objective was to evaluate the fermentation profile, aerobic stability, chemical composition, and mineral content of silages of mango combined with cocoa pod husk meal. A completely randomized design was adopted, including four levels (65, 70, 75, and 80%) and five repetitions, totaling 20 experimental silos that were opened after 90 days of sealing. Increasing mango levels in the silages increased the dry matter recovery, total carbohydrates, and fraction B2 of carbohydrates, and reduced gas losses, dry matter, and mineral matter. The quadratic effect was found for pH, buffering capacity, potassium, boron, iron, and nitrogen fractions A and B1 + B2. Using unconventional products such as mango combined with cocoa pod husk meal for silage making can reduce the cost of food supplementation for ruminants, and the environmental contamination.

Keywords: Aerobic stability. Fermentation profile. Mangifera indica L. Theobroma cacao.

RESUMO
Foi avaliado o perfil fermentativo, estabilidade aeróbia, composição química e conteúdo mineral de silagens de manga combinadas com farelo de casca de cacau. O delineamento adotado foi o inteiramente casualizado, incluindo quatro níveis (65, 70, 75 e 80%) e cinco repetições, totalizando 20 silos experimentais abertos aos 90 dias da vedação. O incremento dos níveis de manga nas silagens aumentou a recuperação de matéria seca, carboidratos totais e fração B2 de carboidratos, e reduziu as perdas de gases, matéria seca e matéria mineral. Foi encontrado um efeito quadrático para pH, capacidade tampão, potássio, boro, ferro e para as frações de nitrogênio A e B1 + B2. A utilização de produtos não convencionais como a manga combinada com a farinha da casca da vagem de cacau para a produção de silagem pode reduzir o custo da suplementação alimentar para ruminantes e a contaminação ambiental.

Palavras-chave: Estabilidade aeróbia. Perfil de fermentação. Mangifera indica L. Theobroma cacao.
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Introduction

The growing interest in ensiling by-products from fruit agribusiness has emerged as an alternative to traditional crops, with the advantage of the low cost of the material to be ensiled. Using these residues for silage making, besides constituting a way of using the materials that would be wasted, contributes to minimizing the impact caused by the accumulation of these residues in the environment (Pinto et al., 2020).

Mango (Mangifera indica L.) is a fruit that generates a high amount of waste during its processing. This fruit contains 74 to 94% of moisture, 13.5 to 21% of carbohydrates, 0.4-0.8% of proteins, and 0.4% of lipids; besides being a source of calcium, phosphorus, iron, potassium, magnesium, and vitamins C, A, B1, and B3 (Sánchez-Santillán et al., 2020). It is estimated that the fruit juice, nectar, and pulp industries generate approximately 40% organic waste, which consists of fresh mango skin, seeds, and bagasse (Jahurul et al., 2015).

The high content of moisture and sugars of mango can negatively affect silage fermentation by allowing the occurrence of undesirable secondary fermentations, leading to a decline in silage quality and losses by effluent drainage from silos (Guzmán et al., 2012). Therefore, to reduce these losses the inclusion of a fiber source, such as the cocoa pod husk meal, could achieve an adequate fermentation.

Cocoa pod husk meal is a fiber-rich feed with a nutritional composition of about 95% of dry matter, 75% of neutral detergent fiber, 69% of acid detergent fiber, and 9.4% of crude protein (Ozung et al., 2016). During harvesting, cocoa pod husk is discarded in the field after the fruit is opened for processing the beans, and is used for fertilizing cocoa trees. However, when the husks remain in the heaped soil, they may become the focus of the transmission of pests and diseases and there may still be excess potassium released in the soil causing an imbalance in plant nutrition. According to Campos-Vega et al. (2018), cocoa pod husk represents on average 67-76% of the total weight of the fruit and can be supplied fresh for ruminant feeding, as silage, or even as a dry meal.

Thus, this study aimed to evaluate the fermentation profile, aerobic stability, chemical composition, and mineral content of silages of mango combined with cocoa pod husk meal.

Material and Methods

The experiment was carried out at the Caatinga Experimental Field, at the Animal Metabolism Unit, belonging to Embrapa Semiárido, located in the municipality of Petrolina, state of Pernambuco, Brazil (latitude 9°8’8.9” S, longitude 40°18’33.6” W, 376 m altitude) whose climate, according to the Köppen & Geiger (1928) classification is the BSw’ semiarid. During the experimental period, the averages of temperature, relative humidity, and evapotranspiration were 26.14 °C, 58.10%, and 4.06 mm, respectively.

Silages were produced with the Palmer mango variety (Mangifera indica L.) and cocoa (Theobroma cacao L.), from fruit growers in the São Francisco Valley region, Brazil. After harvesting and separating the fruits for local trade and export, the mango that would be discarded (the skin and seeds that are discarded during processing, in addition to those whole mangos that did not fit the profile for export) was processed into stationary forage machine (PP-35, Pinheiro Máquinas, Itapira, São Paulo, Brazil) to particles of approximately 2.0 cm. Cocoa pod husk meal was obtained from opening the pod in the field to obtain the beans. Cocoa fruit husks were distributed on a solar dryer with a concrete floor, remaining there to constant weight, reducing its moisture to about 2%. After drying, the material was processed in a forage grinder (TRF 400 Super, 2hp Bivolt - Trapp, Franca, São Paulo, Brazil). Samples of fresh material were collected for further chemical analysis (Table 1).

Table 1 – Chemical composition of mango and cocoa pod husk meal before ensiling

| Variables                      | Cocoa pod husk meal | Mango  |
|-------------------------------|---------------------|--------|
| Dry matter*                   | 872.3               | 137.4  |
| Mineral matter                | 116.6               | 37.6   |
| Organic matter                | 883.4               | 964.0  |
| Ether extract                 | 17.7                | 24.9   |
| Crude protein                 | 123.3               | 90.7   |
| Neutral detergent fiber       | 405.0               | 149.7  |
| Acid detergent fiber          | 304.3               | 121.8  |
| Total carbohydrates           | 743.0               | 846.8  |
| Non-fiber carbohydrates       | 338.0               | 697.1  |

*In g/kg natural matter.
This study used a completely randomized design comprising four treatments and five repetitions per treatment, totaling 20 experimental units. The treatments were: T65% - 65% mango + 35% cocoa pod husk meal; T70% - 70% mango + 30% cocoa pod husk meal; T75% - 75% mango + 25% cocoa meal, and T80% - 80% mango + 20% cocoa meal.

For silage making, mango combined with cocoa pod husk meal was homogenized manually and ensiled in experimental silos made of polyvinyl chloride (PVC), with 10 cm in diameter and 50 cm in height, equipped with a Bunsen valve to allow the gases to escape. At the bottom of the experimental silos, 1 kg of dry sand was protected by a cotton cloth, preventing the contact of the ensiled material with the sand, and allowing effluent drainage. The material was compacted by inserting ± 2 kg of fresh forage per silo. Silos were weighed before and after filling. Once sealed, silos were kept in a covered shed and free from opportunistic animals.

Silos were opened at 90 days, and the ensiled material up to 10 cm from the ends of the silos was discarded. Samples of the ensiled material were collected for further laboratory analysis.

Density (D) of ensiled mass was obtained using the equation of Jobim et al. (2007):

\[ D = \frac{m}{V} \] (1)

where \( m \) = weight of the ensiled material expressed in kg; \( V \) = volume of the ensiled material expressed in kg/m\(^3\).

The volume of the silos was determined using the area and height of the silos, obtaining values expressed in cubic centimeters (cm\(^3\)) and grams (g), which were converted into cubic meters (m\(^3\)) and kilograms (kg), respectively, to express the density in kg/m\(^3\).

Effluent losses (EL, kg/ton of fresh matter) were obtained by the equation:

\[ EL = \left[ \frac{(PSf - Tb) - (PSi - Tb)}{MFi} \right] \times 1000 \] (2)

where \( PSi \) = empty silo weight + sand weight at sealing (kg); \( PSf \) = empty silo weight + sand weight at opening (kg); \( PSa \) = empty silo weight (kg); \( FMi \) = fresh matter at silo sealing (kg).

Gas losses (GL, % dry matter) were obtained by the equation (Amorim et al., 2020):

\[ GL = \left[ \frac{PSf - PSa}{FWf \times DMf} \right] \times 10000 \] (3)

where \( PSf \) = Sealed full silo at sealing (kg), \( PSa \) = weight of the open silo (kg), \( FWf \) = fresh weight (kg) and \( DMf \) = dry weight (%).

Dry matter recovery (DMR, %) of silages was estimated by the equation (Jobim et al., 2007):

\[ DMR = \frac{(DMa \times 100)}{DMf} \] (4)

where \( DMa \) = dry matter content at silo opening (%); \( DMf \) = forage dry matter content at silo sealing (%).

Upon silo opening, the temperature of the silage mass was measured with a digital skewer thermometer (Incoterm 6132, São Paulo - SP, Brazil). The pH of the samples was measured immediately after silo opening, using a portable digital pH meter (Marconi MA-552, Piracicaba - SP, Brazil), previously calibrated. Ammonia nitrogen (NH\(_3\) - N) was determined according to Bolsen et al. (1992). Buffering capacity was determined using a 10 to 20 g fresh sample, expressed in milligram equivalent (e.mg) of alkali, required to raise the pH from 4.0 to 6.0 per 100 g dry matter (Playne & McDonald, 1996).

Aerobic stability was determined according to Kung et al. (2003). Each experimental unit comprised a plastic container with a capacity of 4 L, containing about 2 kg of forage. The containers were kept in a closed room, under a controlled temperature of 24 °C. Values of pH were read at 6-h intervals, totaling 96-h of exposure to air. The temperature was measured at 1-h intervals over the 96-h period.

The variables analyzed were: Maximum TpH = Time to reach the maximum pH (h); MT = maximum temperature (°C); TRMST = Time to reach the maximum silage temperature (h); MDT = Maximum difference in temperature between silage and the environment (°C); SDT = Sum of the differences in temperature between silages and the environment (°C); TIST = Tendency to increase the silage temperature (h), and; AS = Aerobic stability (h).

Collected samples were pre-dried in a forced ventilation oven at 55°C for 72 h and processed in a knife mill (Wiley mill, Marconi, MA-580, Piracicaba, São Paulo, Brazil), using 1 mm sieves. Analyses were carried out using the methods described by AOAC (2016) for the contents of dry matter (DM, method 967.03), mineral matter (MM, method 942.05), crude protein (CP, method 981.10), and acid detergent fiber (ADF; method 973.18). The ether extract content was analyzed using a fat extractor (ANKOM TX-10, Macedon - NY, United States), according to the American Oil Official Method Chemists’ Society (2017). Neutral detergent fiber (NDF) was determined according to van Soest et al. (1991). Total carbohydrates (TC) were estimated using the equation proposed by Sniffen et al. (1992):

\[ TC = 100 - \left( \% \text{ CP} + \% \text{ EE} + \% \text{ MM} \right) \] (5)
Non-fiber carbohydrate (NFC) content was calculated according to Hall (2003):

\[
\%\text{NFC} = \%\text{TC} - \%\text{NDF}
\]  

(6)

Hemicellulose (HEM) was calculated using the following equation:

\[
\text{HEM} = \text{NDF} - \text{ADF}
\]  

(7)

The content of total digestible nutrients (TDN) was estimated by the equation (Undersander et al., 1993):

\[
\%\text{TDN} = 87.84 - (0.70 \times \text{ADF})
\]  

(8)

Total carbohydrates were quantified according to Sniffen et al. (1992), where:

\[
\text{TC (\% DM)} = 100 - (\text{CP} + \text{EE} + \text{MM})
\]  

(9)

and fractionated into A + B1, B2, and C, with non-fiber carbohydrates (NFC) corresponding to fractions A + B1, and obtained by the difference between TC and NDN. Fraction C was obtained by indigestible NDF after 288-h in situ incubation (Valente et al., 2011). Fraction B2, which corresponds to the available fraction of the fiber, was obtained by the difference between NDF and fraction C (indigestible fiber).

The levels of non-protein nitrogen (NPN), neutral (NIDN), and acid (ADIN) detergent insoluble nitrogen were determined according to Licitra et al. (1996). Protein fractionation was calculated using the CNCPs system (Sniffen et al., 1992). Protein was analyzed and calculated for the five fractions, A, B1, B2, B3, and C. Fraction A was obtained by the difference between total nitrogen and trichloroacetic acid (10%) insoluble nitrogen (residual), with the formula:

\[
A (\%\text{Nt}) = \text{Nt} - \text{N1} / \text{Nt} \times 100
\]  

(10)

where \text{Nt} = total nitrogen in the sample and \text{N1} = trichloroacetic acid-insoluble nitrogen.

Fraction B1 (soluble protein rapidly degraded in the rumen) was obtained by the difference between borate phosphate buffer insoluble nitrogen and NPN, by the formula:

\[
B1 (\%\text{Nt}) = \text{N1} - \text{N2} / \text{Nt} \times 100
\]  

(11)

where \text{N2} = borate phosphate buffer insoluble N. Fractions B2 and B3 (insoluble protein with slow intermediate degradation rate in the rumen), were determined by the difference between borate phosphate buffer insoluble nitrogen and NDIN, NDIN minus ADIN, respectively. Fractions B2 and B3 were obtained by applying the equation:

\[
(\%\text{Nt}) = \text{N2} - \text{NDIN} / \text{Nt} \times 100
\]  

(12)

\[
(\%\text{Nt}) = \text{NDIN} - \text{ADIN} / \text{Nt} \times 100
\]  

(13)

Fraction C (insoluble protein indigestible in the rumen and intestine) was determined by the residual N content of the sample after treatment with acid detergent and expressed as a percentage of Nt in the sample.

Concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were determined according to the methodologies described by Nogueira & Souza (2005). Sodium and potassium were determined by flame photometry, whereas the concentrations of Ca and Mg were analyzed by titration, by determining the Ca contents, and subsequently, the Ca + Mg contents, and the Mg content were defined as the difference. Sulfur levels were determined indirectly, first by obtaining the concentrations of sulfates, and subsequently, by considering the atomic molecular weight, the concentration of S was determined. Phosphorus was determined using a molecular spectrophotometer, while the contents of B, Cu, Fe, Mn, and Zn, were determined on an atomic absorption spectrophotometer (model Analyst 100, Perkin Elmer®, Sigma-Aldrich, Germany).

The results obtained were analyzed using PROC GLM of the Statistical Analysis System (SAS University) and tested by analysis of variance and regression at 5% probability. The following statistical model was used:

\[Y = \mu + T_j + e_{ij}\]  

(14)

where \(\mu\) = overall mean; \(T_j\) = effect of inclusion of cocoa pod husk meal; \(e_{ij}\) = residual error.

**Results**

The inclusion levels of mango combined with cocoa pod husk meal in silages had a positive linear effect on the DMR \((P < 0.001)\) and a negative linear effect on GL \((P < 0.001)\). Additionally, a quadratic effect of the inclusion levels of mango combined with cocoa pod husk meal was found for pH \((P = 0.018)\) and buffering capacity \((P = 0.002)\). There was no effect on mango inclusion levels in silages for D, EL, temperature, and NH\(_3\)-N (Table 2).

The inclusion of mango and cocoa pod husk meal had a negative linear effect on DM \((P < 0.001)\) and MM \((P = 0.016)\), and a positive linear effect on TC \((P = 0.021)\) of silage composition. There was no effect on the inclusion levels
of mango in silages for CP, NDF, ADF, EE, NFC, HEM, and TDN (Table 3).

The inclusion levels of mango combined with cocoa pod husk meal showed an increasing linear effect \( (P = 0.019) \) on the carbohydrate fraction B2 of silages but did not affect fractions A + B1 and C (Table 4). A quadratic effect of the inclusion levels of mango combined with cocoa pod husk meal was observed for nitrogen fractions A \( (P = 0.009) \) and B1+B2 \( (P = 0.010) \), but no effect was observed on fractions B3 and C (Table 4).

The exposure of silages of mango with cocoa pod husk meal to the environment did not alter the maximum pH, maximum T pH, MT, TRMST, MDT, SDT, TIST and AE (Table 5).

Regarding the mineral content, the combination between cocoa pod husk meal and mango showed a quadratic effect for K \( (P = 0.046) \), B \( (P = 0.016) \), and Fe \( (P = 0.006) \). Macrominerals N, P, Ca, Mg, S, Na \( (P > 0.05) \) and microminerals Cu, Mn, and Zn \( (P > 0.05) \) were not influenced by increasing levels of mango in silages (Table 6).

**Discussion**

The increase in the DMR content and the reduction in the GL of the silages may be related to the higher levels of soluble sugars in mango converted into lactic acid. According to Matias et al. (2020), the increase in lactic acid, due to the buffering of acids produced by fermentation, prevents the production of ethanol and promotes lower GL and higher DMR.

Using 35% of cocoa pod husk meal in mango silage resulted in a lower buffering capacity. These results are possible because of the greater presence of cations such as K+, Ca2+, and Mg2+ in these silages. These minerals have buffering activity, neutralizing organic acids formed by fermentation, and preventing pH drop (McDonald et al., 1991). However, the decline in pH of the silages may also be related to the greater availability of non-fiber carbohydrates in mango compared to cocoa pod husk. Pahlow et al. (2003) reported that silages with dry matter contents between 30 and 50% can present pH values between 4.35 and 5.00 and be stable after fermentation, which may have occurred in this study.

The decrease in DM and MM contents and the increase in TC content in silages are directly related to the nutritional characteristics of mango with cocoa pod husk (Table 1). Values of dry matter of the silages found in our study are within the range described by...
McDonald et al. (1991) for a good forage fermentation in the silo, which should vary from 28 to 40%. For DM values <28% the silage becomes susceptible to the proliferation of undesirable microorganisms that increase effluent losses, and on the other hand when silages have DM >40% compaction is more difficult, resulting in undesirable air entering the silo.

The increase in the carbohydrate fraction B2 demonstrates an increase in fiber with the potential for degradation. Thus, the increasing inclusion of mango in silages reduces the...
rapidly available energy source, requiring rapidly available energy supplements, in cases where there is no protein restriction in terms of quality and quantity.

During fermentation, the activity of bacteria and enzymes with proteolytic action can modify the nitrogen fractions in silage. The increase in fraction (A) of NPN results from extensive protein hydrolysis, leading to its increase in silage (Ohshima & McDonald, 1978). When mango was included at 80% in silage, the highest content of fraction A was verified, indicating lower content of free amino acids and small peptides (Pinto et al., 2020). Besides the proteolytic action is directly linked to the dry matter content and converting the silage protein into NPN (Silva et al., 2016). Thus, high NPN levels can lead to nitrogen losses if there is no carbon available for microbial protein synthesis.

The N fraction of rapid degradation (B1) and intermediate degradation in the rumen, also known as true protein (B2), is reduced according to the inclusion of mango residue, thus decreasing N availability for the microbial population in the rumen, and interfering with the efficiency of ruminal microbial protein production.

There was no change in the aerobic stability of the silages, which indicated that there was no evidence of deterioration of the silage. According to Borreani et al. (2018), the deterioration process results in heat production, dry matter losses, and pH changes, which have a direct correlation with the quality of the silage.

In general, higher values of mineral elements were verified in silages with a higher proportion of cocoa pod husk, promoted mainly because of the higher content of mineral matter in cocoa pod husk compared to mango, except for boron. This micronutrient is essential for plants and is essential for fruit formation (Dar, 2017), so the higher content of this element in silages with a greater proportion of mango is because of its greater accumulation in mango fruit.

Dietary requirements for K and Fe in cattle are 0.60-0.70 and 40-50%, respectively (National Research Council, 1996). The average values obtained herein are within the appropriate range, suggesting the potential of mango and cocoa pod husk silages for an adequate supply of these minerals. High K values in mango and cocoa pod husk silage were already expected, as fruits are considered a good source of this mineral. Moreover, adequate iron content is important because it is a component of hemoglobin and some enzymes, and its deficiency results in anemia (Katsogiannou et al., 2018).

Conclusion

Using such mango combined with cocoa pod husk meal for silage making can reduce the cost of food supplementation for ruminants, and the environmental contamination.

Conflict of Interest

The authors declare that they have no competing interests.

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