Chemical composition and fermentation characteristics of maize silage with citrus pulp

Composição química e características fermentativas da silagem de milho aditivada com polpa cítrica

SOUZA, Amelina de Oliveira[1]
https://orcid.org/0000-0003-3305-2272

TAVEIRA, José Henrique da Silva[1]
https://orcid.org/0000-0001-9212-1800

SANTOS, Alessandro José Marques[1]
https://orcid.org/0000-0002-6016-0861

FERNANDES, Patrick Bezerra[2*]
https://orcid.org/0000-0003-2368-943X

COSTA, Kátia Aparecida de Pinho[3]
http://orcid.org/0000-0002-9555-5089

COSTA, Carolina Marques[2]
https://orcid.org/0000-0002-0312-6755

GURGEL, Antonio Leandro Chaves[2]
https://orcid.org/0000-0001-5911-369X

SILVA, Ana Carolina Gomes[3]
https://orcid.org/0000-0002-6921-0714

COSTA, João Victor Campos Pinho[3]
https://orcid.org/0000-0002-0948-2603

[1]Universidade Estadual de Goiás, Campus Oeste, Sede São Luís dos Montes Belos, GO, Brazil. campusoeste.ueg.br/
[2]Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. ufms.br
[3]Instituto Federal de Educação, Ciência e Tecnologia Goiano, GO, Brazil. ifgoiano.edu.br/
*Mail for correspondence: zoo.patrick@hotmail.com

ABSTRACT

The objective of this study was to evaluate the effects of including citrus pulp in maize silage on chemical composition and fermentation parameters. The experimental design was fully randomized with four replicates. Maize silage consisting of 0–50% citrus pulp was produced and analyzed. The pH linearly decreased with the increased inclusion of citrus pulp (3.49 at 50% citrus pulp), which is far below what is considered adequate (3.8–4.2). However, not all fermentation parameters were compromised. At 33% citrus pulp, we estimated 30.82 g/kg total nitrogen. At 49% citrus pulp, we estimated 95.16 g/kg of crude protein. At 21% and 22% citrus pulp, we estimated 549.89 g/kg of neutral detergent fiber and 678.11 g/kg of total digestible nutrients, respectively. Therefore, we conclude that the inclusion of up to 30% citrus pulp improves the complete chemical composition of maize silage owing to the reduction in structural carbohydrate values and increases in total digestible nutrient and protein content.
Keywords: Agribusiness**,** co-product**,** crude protein, fermentation parameters**,** mixed silage.

RESUMO

O objetivo deste trabalho foi avaliar o efeito da inclusão de polpa cítrica na silagem de milho sobre a composição química e parâmetros de fermentação. O delineamento experimental utilizado foi o inteiramente casualizado, com quatro repetições. Os tratamentos foram: Silagem de milho sem polpa cítrica (0%); silagem de milho com 10% 39 de polpa cítrica; silagem de milho com 20% de polpa cítrica; silagem de milho com 30% de polpa cítrica; silagem de milho com 40% de polpa cítrica e silagem de milho com 50% de polpa cítrica. O pH diminuiu linearmente com à inclusão de polpa cítrica, gerando valores distantes do considerado adequado (3,8 a 4,2). Por outro lado, é possível inferir que nem todos os parâmetros fermentativos foram comprometidos, uma vez que, com a inclusão de 33% de polpa cítrica, é possível estimar 30,82 g/kg total N. Com a utilização 45 de 49% de polpa cítrica, é possível obter 95,16 g/kg de proteína bruta. De 21% a 22% de polpa cítrica, foi obtido 549,89 g/kg de fibra em detergente neutro e 678,11 g/kg de nutrientes digestíveis totais. A inclusão de até 30% de polpa cítrica melhorará significativamente a composição química da silagem de milho, devido à redução nos valores de carboidrato estrutural e aumentos nos nutrientes digestíveis totais e proteína.

Palavras-chaves: Agroindústria, coproduto, proteína bruta, parâmetros de fermentação, silagem mista.

INTRODUCTION

Maize (*Zea mays* L.) agriculture is of great economic importance to many tropical climate regions (*e.g*., Brazilian savannah, locally called "cerrado") where maize is a dietary staple for both humans and domestic animals (Ferreira et al., 2016; Pavan & Duckett, 2019).

One strategic use of maize is the fermentation of the aerial parts (leaf blade, stem, ear, grain) to create preserved biomass (silage) for the nutrition of domestic ruminants. One can easily observe fermentation parameters in the ensilaging process (low acidity and ammonia nitrogen), and the process leads to adequate *in vitro* dry matter digestibility values of 65%, and total digestible nutrients of 66% (Zardin et al., 2017).

Nevertheless, maize plants and other grasses grown in tropical climates naturally contain high levels of structural carbohydrates and low levels of proteins and fatty acids (Oliveira et al., 2020;

Paludo et al., 2020; Oliveira et al., 2021). Therefore, it is necessary to use assistive strategies to improve the chemical composition of maize silage without compromising the storage process of ensiled material.

One option for improving the chemical composition of maize silage is enrichment with natural additives and/or agribusiness residues, as they usually have a low acquisition cost. Therefore, a potential strategy would be the use of co-products from the juice industry such as citrus pulp or peel. Ítavo et al. (2020) observed that completely replacing maize silage in the diet of dairy cows with orange (*Citrus sinensis*) peel silage did not cause adverse effects in the rumen environment. Additionally, the high proportion of dietary fatty acids in citrus pulp provide promising results in increasing milk fat content (Leite et al., 2017).

However, it is still necessary to carry out more research to understand the limits and implications of the inclusion of

citrus by-products in maize silage. Pure citrus pulp does not have an ideal fermentation profile because of its high moisture content (Ülger et al., 2020). Thus, the objective of this study was to evaluate the effects of citrus pulp in maize silage on chemical composition and fermentation parameters of maize silage.

The following hypotheses were tested: (1) Inclusion of up to 50% citrus pulp improves the chemical composition of maize silage. (2) A high proportion of citrus pulp is positively correlated with improved chemical composition of maize silage.

MATERIALS AND METHODS

Experimental area information

The study was carried out on a school farm belonging to the State University of Goiás West Campus, São Luís de Montes Belos, GO (16º 32' 23.60" S, 50º 25' 11.77" W). The average altitude was 542 m. The experiment was conducted between April 2018 and July 2018.

The maize used in the experiment was obtained from Esperança farm, São Luís de Montes Belos, GO (17° 44' 02.1" S, 51° 13' 58.3" W).

Citrus pulp was supplied by a private orange juice processing company located in Goiânia, GO, Brazil.

Experimental design

The experimental design was fully randomized with four replicates. The treatments were: maize silage without citrus pulp (0%); maize silage with 10% citrus pulp; maize silage with 20% citrus pulp; maize silage with 30% citrus pulp; maize silage with 40% citrus pulp; and maize silage with 50% citrus pulp.

Maize

The maize variety used was Nidera NS90. The crop was fertilized with 150

kg potassium chloride/ha, 150 kg monoammonium phosphate/ha, and 200 kg urea/ha. Maize was harvested on April 1, 2018. Upon harvest, the crop had 34% dry matter (DM) and hard kernels with a 50% milk line.

According to Köppen's classification, the climate of the study region is Aw (tropical savanna with dry winter), with an average temperature of 23.5 °C, ranging from 20.7 °C in June to 25.0 °C in December. The average annual precipitation is 1,785 mm, of which 87% is concentrated between October and March. The region has an average rainfall deficit of four months (Alvares et al., 2014).

Citrus pulp

The citrus pulp collected from the agroindustrial unit had a water content of 70%. The pulp was placed on a plastic sheet approximately 10 cm high and sun-dried for approximately 8 h at 29 °C while being turned over with a rake every 2 h to ensure even drying. At the time of ensilage, the citrus pulp had a DM content of 35%.

Preparation and analysis of silage

Ensilage was carried out on the Córrego São Pedro farm (16° 31' 24" S, 50° 25' 31" W). Data analysis was performed at the State University of Goiás São Luís de Montes Belos Campus Laboratory of Bromatology and at the Goiano Federal Institute Rio Verde Forage Laboratory (17° 48' 11" S, 50° 54' 22" W).

For silage production, whole maize plants were harvested from above the first node and crushed into particles of 3–5 cm using a stationary forage harvester. Citrus pulp was milled for forage to reach a particle size of 5 cm. Samples of fresh maize and citrus pulp were set aside for nutritional analysis, while the rest was ensiled.

Maize was ensiled in 24 batches, with four repeats of each of the six treatments (0, 10, 20, 30, 40, and 50% citrus pulp). Each batch was stored in an experimental PVC silo (measuring 10 cm in diameter, 50 cm in length, and 3925.000 cm^3 in volume), compacted, closed with a PVC cover, and sealed with an adhesive tape to prevent the entry of air. The samples were then stored at room temperature and protected from rain and sunlight for 65 days.

For chemical analysis of fresh maize and citrus pulp, samples were pre-dried in a forced-ventilation oven at 55 °C for approximately 72 h and then ground in 1 mm sieves. DM, ash (MM), crude protein (CP), and ether extract (EE) contents were determined according to AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to Mertens (2002). Total digestible nutrients (TDN) content was estimated using the equation (TDN g/kg DM = 105.2 – 0.68*(NDF g/kg DM)) proposed by Chandler (1990) (Table 1).

Table 1. Bromatological analysis of maize and citrus pulp before ensiling.

Item (g/kg DM)	Maize	Citrus pulp
Dry matter [1]	336.20	348.80
Crude protein	77.40	109.60
Ether extract	29.90	47.10
Neutral detergent fiber	477.30	368.70
Acid detergent fiber	249.60	220.90
Ash	51.00	368.00
Total digestible nutrients	727.40	801.30

[1] g/kg of natural matter

After 65 days of fermentation, the silos were opened, and the top and bottom of each silo were discarded. The central portion of each batch of silage was homogenized and placed in a plastic tray. Approximately, 0.5 kg of each batch of silage was placed in a forced ventilation oven at 55 °C for approximately 72 h, ground in a knife mill with a 1 mm sieve, and placed in a plastic container. The chemical characteristics were analyzed using the same methods as those described for the fresh material.

In vitro dry matter digestibility (IVDMD) was determined according to the technique described by Tilley and Terry (1963), which was adapted to the artificial rumen developed by ANKON®, using the "Daisy incubator" instrument from Ankom Technology (*in vitro* true digestibility, IVTD).

Another aliquot of the sample was separated for analysis of the fermentation parameters. We measured pH, ammonia nitrogen in relation to total nitrogen (N-NH$_3$ g/total kg N), and buffer capacity (BC eq.mg HCL/100 g DM) using the methods described by Silva and Queiroz (2002).

Statistical analysis

Data related to citrus pulp levels were analyzed following a completely randomized model ($Y_{ij} = \mu + C_i + \varepsilon_{ij}$); Y_{ij}, observed value; μ, general constant; C_i, effect of citrus pulp levels (i = 0%, 10%, 20%, 30%, 40%, and 50%); j, repetition effect (j = I, II, II, IV); ε_{ij}, random error associated with each observed value.

The levels of citrus pulp were subjected to regression analysis of first-degree ($Y_{ij} = \beta0 + \beta1 * X + \varepsilon_{ij}$) and second-degree ($Y_{ij} = \beta0 + \beta1 * X + \beta2 * X^2 + \varepsilon_{ij}$); Y_{ij}, observed value; $\beta0$, equation intercept; $\beta1$ and $\beta2$, slope of the equation; X, effect of citrus pulp levels (0%, 10%, 20%, 30%, 40%, and 50%); ε_{ij}, error associated with the response variable in the model that exhibited a significant effect at 5% and the highest coefficient of determination (R^2).

To carry out the analyses, the ExpDes package (Ferreira et al., 2014) of the R software version 4.0 was used.

RESULTS

The inclusion of citrus pulp in maize silage influenced all three fermentation parameters as follows: pH ($p = 0.018$), $N-NH_3$ ($p < 0.001$), and BC ($p < 0.001$) (Table 2).

The pH decreased linearly with increasing proportion of citrus pulp, allowing us to confirm that as the proportion of citrus pulp increases, the acidity of the ensiled material increases (Table 2).

Regarding the other fermentation parameters ($N-NH_3$ and BC), the best fit was observed with the second-degree equation, indicating a parabolic relationship to citrus pulp content. Therefore, we estimate a minimum $N-NH_3$ value of 30.82 g/kg at 33% citrus pulp and a minimum BC value of 23.24 eq.mg HCL/100 g MS at 46% citrus pulp (Table 2).

Table 2. Fermentation parameters of maize silage with inclusion of increasing levels of citrus pulp.

Variable	Inclusion of citrus pulp (%)						C.V (%)	Equation	R²		P - value	
	0	10	20	30	40	50			L	Q	L	Q
pH	3.67	3.69	3.63	3.61	3.49	3.49	2.53	$Y = 3.70 - 0.004 \cdot X$	0.886	0.915	<0.001	0.473
N-NH$_3$ (g/kg)	56.82	34.22	34.55	35.15	32.00	34.62	17.81	$Y = 52.93 - 1.33 \cdot X + 0.020 \cdot X^2$	0.449	0.793	<0.001	0.001
BC (eq.mg HCL/100 g DM)	34.65	27.55	27.11	25.13	24.97	23.76	10.89	$Y = 33.59 - 0.455 \cdot X + 0.005 \cdot X^2$	0.765	0.910	<0.001	0.037

C.V: coefficient of variation. R²: coefficient of determination. L: linear regression. Q: quadratic regression. Y: dependent variable (fermentation parameters). X: citrus pulp levels (0%, 10%, 20%, 30%, 40%, and 50%). N-NH$_3$: ammonia nitrogen. BC: buffering capacity.

The increase in citrus pulp proportion affected the chemical composition of the silage in the following parameters: CP ($p < 0.001$), NDF ($p = 0.001$), ADF ($p < 0.001$), EE ($p = 0.002$), MM ($p < 0.001$), and TDN ($p = 0.001$) contents. Citrus pulp inclusion had no effect ($p > 0.05$) on DM and IVDMD (Table 3).

The effect of increasing citrus pulp proportion on CP, NDF, ADF, and TDN contents adjusted the quadratic equation, with R^2 ranging from low to moderate. Thus, with the use of 48–49% of citrus pulp, 291.94 g/kg ADF and 95.16 g/kg CP were measured. Using only 21–22% citrus pulp, 549.89 g/kg NDF and 678.11 g/kg TDN (Table 3) were measured.

EE and ash contents increased linearly with increasing citrus pulp level. Thus, we infer that higher citrus pulp concentrations results in a higher concentration of inorganic matter and possible improvements in the fatty acid profile of silage (Table 3).

Table 3. Chemical composition of maize silage including increasing levels of citrus pulp.

Composition	Inclusion of citrus pulp (%)						C.V (%)	Equation	R²		P -value	
	0	10	20	30	40	50			L	Q	L	Q
DM (g/kg)	336.20	364.50	341.62	335.22	337.17	348.47	2.31	$Y = 346.64$	0.016	0.020	0.426	0.698
CP (g/kg DM)	77.42	78.20	78.62	98.20	98.62	88.32	6.62	$Y = 73.60+0.881*X - 0.009*X^2$	0.522	0.595	<0.001	0.049
NDF (g/kg DM)	477.40	605.62	529.40	521.40	513.80	488.62	7.05	$Y = 509.63+ 3.74*X - 0.087*X^2$	0.072	0.354	0.157	0.009
ADF (g/kg DM)	249.60	294.30	285.35	270.62	287.42	296.10	3.38	$Y = 261.89 + 1.25*X - 0.013*X^2$	0.356	0.401	<0.001	0.093
MM (g/kg DM)	37.50	41.42	46.00[ab]	42.97	44.62	51.97	6.13	$Y = 38.44 + 0.225 *X$	0.755	0.758	<0.001	0.666
EE (g/kg DM)	29.92	24.60	34.50	31.02[ab]	34.12	32.50	9.92	$Y = 28.40 + 0.108*X$	0.310	0.324	0.008	0.546
TDN (g/kg DM)	727.37	640.17	692.01	697.44	702.61	719.73	3.60	$Y = 705.45 - 2.54*X + 0.059*X^2$	0.072	0.354	0.157	0.009
IVDMD (g/kg DM)	588.94	613.44	614.32	646.23	700.74	677.43	9.14	$Y = 587.59$	0.850	0.851	0.928	0.670

C.V: coefficient of variation. R²: coefficient of determination. L: linear regression. Q: quadratic regression. Y: dependent variable (composition). X: citrus pulp levels (0%, 10%, 20%, 30%, 40%, and 50%). DM: dry matter. CP: crude protein. NDF: neutral detergent fiber. ADF: acid detergent fiber. EE: ether extract. MM: ash. IVDMD: *in vitro* digestibility of dry matter. TDN: total digestible nutrients.

DISCUSSION

The evaluation of pH, N-NH$_3$, and BC provides an overview of the fermentation process that occurs during the storage of ensiled biomass (Kung et al., 2018; Santos et al., 2020). After the storage period of maize silage containing citrus pulp, the pH values did not approach what is considered adequate (3.8 to 4.2), as recommended by McDonald et al. (1991) and Tomich et al. (2004).

On the other hand, the inclusion of up to 33% citrus pulp did not generate high values of ammonia nitrogen; despite the reduction in the fraction of maize in the ensiled material, values far below 100 g/kg of total N were observed. Providing adequate lactic fermentation reduces proteolysis and inhibits the growth of unwanted microorganisms (Kung and Shaver, 2001). However, in this study, which aimed to include up to 50% of citrus pulp in maize silage, the ensiled material could not be considered satisfactory or desirable, as the BC values were not adequate. Ideally, values below 20 eq.mg HCL/100 g MS are recommended to obtain quality silage (Souza et al., 2019).

Regarding the chemical composition, the inclusion of 49% citrus pulp promoted a 26% increase in CP content to 95.16 g/kg. This value exceeded the minimum recommended CP content (70.0 g/kg DM), ensuring the maintenance of the ruminal microbiota (Van Soest, 1994). As noted by Ítavo et al. (2020), we confirm that the inclusion of orange pulp in corn silage can be considered an acceptable alternative in replacement levels.

The increase in EE content with increasing citrus pulp content suggests that citrus pulp increases the energy density of silage (Table 3). Fresh citrus pulp has higher energy density and TDN values, and lower NDF and ADF values, than fresh maize (Table 1). According to Ülger et al. (2020), these features of citrus pulp will promote improvements in maize silage, owing to the potential increase in non-fibrous carbohydrate content.

It should be emphasized that the MM content only indicates the total mineral content of the sample. High levels of MM can be caused by high levels of silica, which cannot be used by animals (Santos et al., 2020).

Regarding IVDMD, the values observed in mixed silage (Table 3) are lower than those observed by Zardin et al. (2017), suggesting that the inclusion of 50% citrus pulp did not favor significant improvements in the digestibility of the ensiled material. Furthermore, the observed IVDMD values of silage containing citrus pulp were not significantly different from those of 100% maize silage.

CONCLUSION

The inclusion of up to 30% citrus pulp will improve the complete chemical composition of maize silage due to the reduction in structural carbohydrate values and increases in TDN and protein contents.

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