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Effect of storage time on the chemical composition of whole and grainless corn plant silage harvested at different maturity stages

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ABSTRACT - The objective of this study was to evaluate the effect of harvesting corn at different maturity stages for silage production combined with four storage periods, considering nutrient loss and the chemical composition of silage of the whole plant and also of grainless plants. The experiment was performed in a completely randomized design with a factorial arrangement of six maturity stages of corn [reproductive (R); R1 to R6] and four periods of silage storage (30, 60, 90, and 120 days). At stages R1 and R2, losses by effluents occurred more intensely when compared with the more advanced stages, whereas losses by gases showed the opposite behavior, with stage R6 responsible for the greatest losses. Stage R4 showed a stable dry matter (DM) content during the storage period, 42.08%, and had the lowest levels of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) for the whole plant silage. The ADL in the silage of grainless plant of stage R4 was not the lowest, but it was close to the first reproductive stages, being an important point, since the first reproductive stages can present a vegetative fraction of better quality. The highest DM volumes produced by area occurred in the most advanced stages; however, R5 obtained a greater reduction in volume over the days of storage (20.66 kg day^{-1}). The production cost was lower at the advanced stages (R4, R5, and R6) when compared with the first reproductive stages (R1, R2, and R3), and in general, during the storage period the costs did not have great variations. Based on the chemical composition of the silages, DM losses during the storage period, the stability in the costs of DM ton over the days of storage, the R4 stage was the most suitable for silage production.

Keywords: chemical composition, fermentation process, loss

1. Introduction

Preservation of forage as silage is a widespread practice worldwide, whose main objective is to maintain the nutritional quality of the feed as close to natural as possible, ensuring the year-round supply of quality feed to meet the requirements of animals, and contribute to improvements in animal production (Machado et al., 2011; Macêdo et al., 2019).

Corn is the most used forage plant for making silage, as it has an adequate content of soluble carbohydrates, low buffering capacity, and a balanced epiphytic flora, essential for the occurrence of an adequate fermentation (Neumann et al., 2018; Rabelo et al., 2018). These characteristics are easily modified by the maturity stage at which the crop is harvested, since there are changes in the proportion and quality of the structural components of the plant, changing the final quality of the feed (Zopollatto et al., 2009; Santos et al., 2014).

The maturity stage, in addition to providing structural changes in the plant, also influences the biochemical and biological reactions during the silage storage period. Dry matter (DM) content below 30% predisposes the production of effluents and the growth of clostridial bacteria, whereas contents above 37% can hinder the compaction and expulsion of oxygen from the ensiled mass, favoring the growth of yeasts (Jobim et al., 2007; Muck, 2010).

In addition to harvest maturity, silage storage time also promotes changes in its quality; however, the magnitude of these changes is not fully elucidated. Silages stored for long periods may present reduced concentrations of soluble carbohydrates, as they are used by microorganisms as a substrate during fermentation, increasing the concentrations of fiber compounds (Pedroso et al., 2005; Neumann et al., 2007). In agreement, Senger et al. (2005) reported that the increase in storage time modifies the fibrous structure of the silage, due to acid digestion that occurs during the fermentation process, which goes beyond 21 days (Young et al., 2012).

Although there are studies that evaluate these parameters in corn silage, little is known about the effect of the fermentation process and storage time on the vegetative portion of the plant, which in the present study is characterized by silage of plants without grains. In this context, the present study aimed to evaluate the effect of harvesting corn at different maturity stages for silage production, combined with four storage times, on losses and chemical composition of whole plant and grainless plant silages.

2. Material and Methods

The experiment was conducted in Guarapuava, PR, Brazil (25°23'36" S, 51°27'19" W, with 1,100 m altitude). The climate of the region is humid subtropical mesothermal (Cfb), with no dry season, with cool summers and moderate winter, according to the Köppen classification. Figure 1 shows the maximum and minimum temperatures (°C) and rainfall in mm during the experimental period (2017/2018).

The soil of the experimental area is classified as Typical Bruno Latosol. The area where corn was grown has been used in recent years with annual cycle pastures in the winter, and corn crops in the summer, receiving phosphorus and potassium fertilizations at each growing season, according to the Fertilization and Liming Recommendations for the state of Paraná (SBCS/NEPAR, 2017).

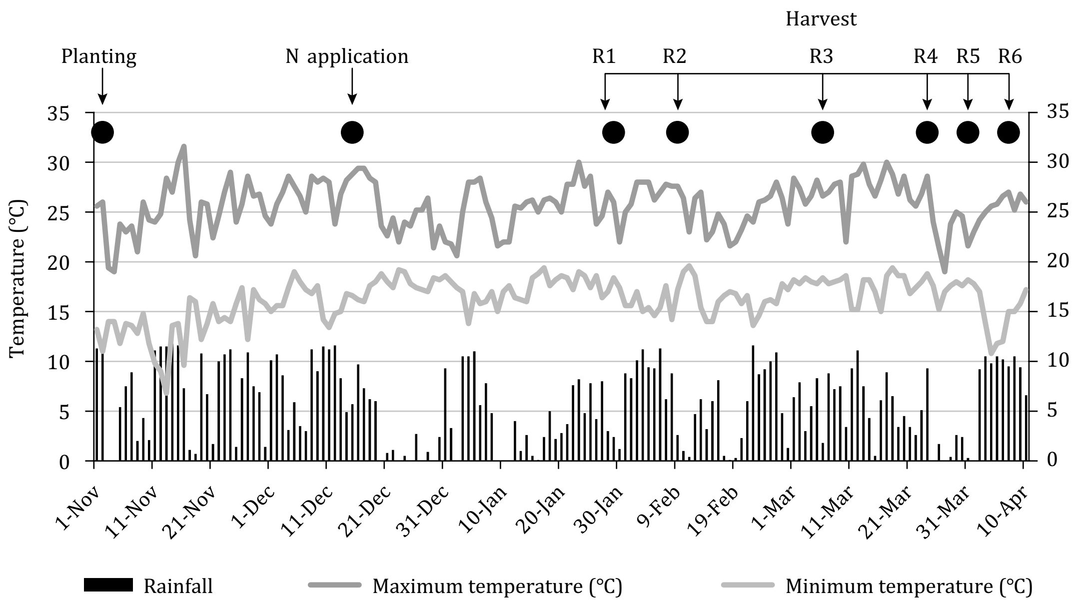

Figure 1 - Maximum and minimum temperatures (°C) and rainfall (mm) during the experimental period (2017/2018).

Corn was planted on November 2, 2017 in a no-till system, using 0.80-m row spacing, 0.04-m sowing depth, and distribution of five seeds per linear meter, aiming at a final population of 62.500 plants ha^{-1}. The hybrid used was Maximus Viptera 3 (Syngenta®), early cycle, with dual purpose for the production of grains and silage, with hard grains and Glyphosate resistance.

Basal fertilization was performed with 500 kg ha^{-1} of the fertilizer 12-31-17 (N-P_2O_5-K_2O). Topdressing fertilization was carried out with 500 kg ha^{-1} of the commercial fertilizer Yara Bela Plus®, with a formulation of 27% N, 5% Ca, 3.7% S, at the vegetative stage 5 (V5). Before sowing, the area was managed considering the preventive control of weeds and insects, by the chemical method using the herbicide based on Glyphosate (commercial product Roundup WG®: 2 kg ha^{-1}) and Imidacloprid + Beta-cyfluthrin (commercial product Connect®: 0.75 L ha^{-1}) plus mineral oil (commercial product Nimbus®: 0.5 L ha^{-1}), and for post-emergence control, Atrazine + Simazine (Primatop® commercial product: 3 L ha^{-1}) and Nicosulfuron (commercial product Nortox®: 0.7 L ha^{-1}) plus Alpha-cypermethrin (commercial product Imunit®: 0.18 L ha^{-1}), as required by the crop, respectively.

Corn plants were harvested at their reproductive stages, as determined by Ritchie et al. (2003), who use the letter R to determine that it is the reproductive stage, and this letter is followed by a number, which indicates the stage, from 1 to 6 [stage R1 – start of grain filling, 88 days after emergence; stage R2 – milky grain, 99 days after emergence; stage R3 – pasty grain, 124 days after emergence; stage R4 – hard dough grain, 142 days after emergence; stage R5 – dent grain, 149 days after emergence; and stage R6 – grain at physiological maturity, 156 days after emergence] combined with four storage times (30, 60, 90, and 120 days), both for whole plant and for grainless plants. The experimental design was a 6 × 4 factorial completely randomized, totaling 24 treatments and four repetitions, totaling 96 PVC silos. Plants for making silages were found in an area of 230.40 m^2 and were randomly harvested according to each stage of maturation evaluated. To harvest the material at the right time, continuous monitoring was carried out at two-day intervals regarding the progress of maturity of corn and for the silo unloading at the correct date, a schedule for opening the silos was prepared according to each treatment.

Corn plants present in the useful area were harvested manually 20 cm from the ground, after which they were minced with the help of a stationary silage chopper (Nogueira PN-Plus 2000) to chop particles between 8 and 12 mm. It is noteworthy that the particle size evaluation at the time of the equipment adjustment was done through the Penn State Particle Size Separator; chopping was performed only from the vegetative portion of the plants. Grains were threshed manually and kept intact for later homogenization with the vegetative portion of the plant for subsequent silage. In all treatments and repetitions, care was taken to never add grains at random, so as not to increase their proportion. Sequentially, the chopped material was ensiled in PVC silos, 50 cm high and 10 cm in diameter. The material was compacted by a press, standardizing the specific mass to 700 kg natural matter m^3. Silos were sealed with a shade screen and a 100-micron plastic package, where a space was left for the accumulation of eventual effluents produced by the ensiled mass. Effluents were drained daily with the aid of a syringe and quantified with a graduated cylinder.

A 500-g sample of the whole corn plant and grain-free plant from each stage of maturation and in fresh form was collected and allocated in a forced-air oven at 55 °C. After 72 h, samples were weighed again to determine the DM content, according to AOAC (1995). In addition to the quantification of the DM content of the raw material, the proportion of grains present in each maturity stage was also determined (Table 1).

Gas losses were quantified in the silages of all maturity stages at 30, 60, 90, and 120 days of storage, using the equation proposed by Schmidt (2006):

$$G = \left(\frac{((PCen - Pen) * MSen) - ((PCab - Pen) * MSab)}{((PCen - Pen) * MSen)} \right) \times 100, \quad (1)$$

in which G = gas loss in % DM; PCen = silo weight at ensiling (kg); Pen = weight of empty silo at ensiling (kg); MSen = DM content of forage at ensiling (%); PCab = weight of the full silo at the opening (kg); and MSab = DM content of forage at the opening (%).

Table 1 - Average dry matter (DM) contents of the whole plant and grainless plant harvested at different maturity stages and participation of grains in the plant structure

Variable	Corn maturity stage					
	R1	R2	R3	R4	R5	R6
Whole plant DM (% DM)	15.06	16.65	23.60	31.84	36.81	45.44
Grainless plant DM (% DM)	14.05	15.65	22.22	24.58	26.05	30.47
Grain DM (% DM)	7.01	11.46	47.77	65.14	66.53	69.00
Grains in the plant (% DM)	0.40	4.09	36.05	48.81	49.27	54.00

R - scale of definition of the maturity stage of the corn plant, described by Ritchie et al. (2003).

Upon opening the silos, silage pH was determined according to the methodology established by Cherney and Cherney (2003). Two samples of 500 g were also collected homogeneously and pre-dried in a forced-air oven at 55 °C. After 72 h, they were weighed again to determine the DM content according to AOAC (1995). After weighing, grains from one of the samples were removed manually to characterize the ensiled grainless plant and thus verify the effects that the fermentative process promotes on the fibrous portion of the plant. Subsequently, all were ground in a Wiley mill, with a 1-mm mesh sieve.

In the samples of whole and grainless plant, contents of neutral detergent fiber (NDF) were determined according to Van Soest (1994), using α thermostable amylase (Termamyl 120L, Novozymes Latin América Ltda.). Acid detergent fiber (ADF) content was determined according to Goering and Van Soest (1970), crude protein (CP) by the micro Kjeldahl method, and acid detergent lignin (ADL) as described by Silva and Queiroz (2009).

Concentrations of non-fiber carbohydrates plus ether extract (NFC + EE) were calculated according to the equation of Henriques et al. (2007):

$$\mathrm{NFC + EE = 100 - (CP + MM + NDF)} \tag{2}$$

The economic analysis was performed through the cost of planting and managing the crop (R\$ ha^{-1}) on the average DM production expressed in kg of DM ha^{-1} of silage in each maturity stage after their respective maturation times storage. For calculation, the fixed costs of the property were not considered. Costs of seeds already treated with insecticide, fertilizers, herbicides for pre-planting desiccation, selective post-emergence herbicide, insecticide, and mineral oil were accounted for at the time of purchase. Therefore, the costs related to machine hours and daily employee hours were obtained from data provided by the ABC foundation. These calculations were made after all the crop management. Silage production, in ton DM ha^{-1}, was calculated through the production of forage DM ha^{-1} subtracting DM losses during the fermentation process of each maturity stage, according to the equation of Schmidt (2006):

$$\mathrm{DML} = \left(\frac{(\mathrm{DMi - DMf})}{(\mathrm{DMi})}\right) \times 100, \tag{3}$$

in which DML = total loss of DM; DMi = initial DM amount [silo weight after filling – weight of the empty set, without forage, before filling (dry tare) × DM content of forage in the silage]; and DMf = amount of final DM [weight of the filled silo before opening – weight of the empty set, without forage, after opening the silos (wet tare) × forage DM content at the opening]. From this result, it was possible to determine the production of silage in ton DM ha^{-1} and the cost of ton DM^{-1} after ensiling.

Data were subjected to Shapiro-Wilk and Bartlett tests to check the assumptions of normality and homogeneity of variance, respectively. Once these assumptions were met, the F test was applied at 5% probability, through Analysis of Variance (ANOVA) and then the Tukey's test for comparison of multiple means at 5% significance.

The analysis of each variable followed the statistical model:

$$\hat{Y}ij = \mu + Si + Tj + (S \times T)ij + \mathcal{E}ij, \tag{4}$$

in which $\hat{Y}ij$ = dependent variables, μ = overall mean of all observations, Si = effect of maturity stage of corn of order "i", Tj = effect of storage time of order "j", (S × T)ij = effect of the interaction between maturity stage of corn of order "i" and storage time of order "j", and Ɛij = residual random effect.

Data collected were also subjected to polynomial regression analysis, using the procedure "proc reg" of SAS (Statistical Analysist System, 1993) to evaluate its behavior during the silage storage period.

3. Results

Effluent and gas losses, as well as the DM content of the silages, presented an interaction ($P<0.05$) between maturity stage and storage time, as well as the chemical parameters of the silages of the whole corn plant and grainless corn plant. However, for variables pH and NFC + EE of the ensiled grainless corn plant, there was no interaction ($P>0.05$).

The advancing of the storage period at stages R2, R3, R5, and R6 (Table 2), caused the silage DM levels to tend to increase linearly at stages R2, R3, and R5, increasing the DM by 0.04, 0.04, and 0.06% day^{-1},

Table 2 - Dry matter (DM) content, pH, production of effluents (L ton dry matter day^{-1}), and losses as gases of corn silages prepared at different stages of maturity according to different storage times

	Storage time (days)				Regression equation $\hat{Y}$ =	CV (%)	R^2
	30	60	90	120			
	% DM						
R1	22.46	22.43	22.40	22.37	22.41^{ns}	3.12	0.004
R2	20.92	22.12	23.32	24.52	19.72 + 0.04D*	7.19	0.44
R3	29.05	30.25	31.45	32.65	26.37 + 0.04D*	3.61	0.65
R4	37.89	37.71	37.53	37.35	37.62^{ns}	2.28	0.07
R5	32.48	34.28	36.08	37.88	30.68 + 0.06D*	2.58	0.84
R6	48.69	47.79	46.89	45.99	49.49 – 0.03D*	1.47	0.77
	pH						
R1	3.7	3.8	3.8	3.8	3.70 + 0.001D*	2.14	0.28
R2	3.7	3.7	3.8	3.8	3.67 + 0.001D*	1.55	0.43
R3	3.8	3.9	3.9	3.9	3.9^{ns}	2.31	0.18
R4	3.9	4.0	4.0	4.0	4.0^{ns}	1.24	0.32
R5	4.0	4.0	4.1	4.1	4.0^{ns}	3.18	0.02
R6	4.1	4.1	4.1	4.1	4.1^{ns}	1.70	0.01
	Effluent production (L ton DM^{-1})						
R1	36.62	40.52	44.42	48.32	32.72 + 0.13D*	11.26	0.49
R2	36.78	43.68	50.58	57.48	29.88 + 0.23D*	10.80	0.72
R3	3.59	5.09	6.59	8.09	2.09 + 0.05D*	42.69	0.32
R4	0.14	0.74	1.34	1.94	−0.46 + 0.02D*	52.67	0.62
R5	0.46	0.73	1.00	1.27	0.19 + 0.009D*	36.40	0.51
R6	-	-	-	-	ND		
	Losses as gases (%)						
R1	6.32	6.26	6.20	6.14	6.23^{ns}	17.24	0.006
R2	6.13	6.22	6.31	6.40	6.27^{ns}	12.81	0.01
R3	5.49	5.46	5.43	5.40	5.44^{ns}	14.65	0.002
R4	7.26	7.32	7.38	7.44	7.35^{ns}	10.93	0.01
R5	10.83	11.01	11.19	11.37	11.10^{ns}	13.39	0.02
R6	27.1	28.9	30.70	32.50	20.63 + 0.06D*	6.68	0.62

R - scale of definition of the maturity stage of the corn plant, described by Ritchie et al. (2003); D - storage days; CV - coefficient of variation; ND - not detected; R^2 - coefficient of determination.
* $P<0.05$ and ns - non-significant by Tukey's test.

respectively, while at the R6 stage there was a linear decreasing trend, reducing the DM by 0.03% day^{-1}. However, only at the maturity stages R5 and R6, the DM content of the silage was close to that of fresh forage (Table 1), a behavior described in the literature as desirable.

The pH of the silages produced in the different maturation stages increased ($P<0.05$) at stages R1 and R2 (Table 2) in the order of 0.001 pH point day^{-1}, while in the other stages, they remained stable ($P>0.05$).

The DM content of the plant at harvest is closely related to the volume of effluents produced and losses as gases. In view of this, it can be seen that the production of effluents was more intense when the plants were harvested at stages R1 and R2 (0.13 and 0.23 L ton DM day^{-1}, respectively). As the harvest stages advanced to R3, R4, and R5, effluent production continued ($P<0.05$), but to a lesser extent (0.05, 0.02, and 0.009 L ton DM day^{-1}, respectively) (Table 2).

Losses as gases were lower in the early maturity stages and increased gradually. When analyzing the storage days, only the R6 stage showed significant changes ($P<0.05$) during the storage period, increasing losses linearly by 0.06% day^{-1}.

The NDF values of the whole ensiled plant increased linearly at stages R1 (0.01% day^{-1}) and R2 with quadratic tendency, reached its highest value at 77 days of storage (69.95%), whereas at the other stages, it did not show significant differences during the storage period (Table 3).

In the evaluation of ensiled grainless plants (Table 3), only stages R1, R3, and R5 underwent changes in NDF content during the storage period ($P<0.05$). In a linear fashion, the stage R1 increased by 0.13% day^{-1}, whereas stages R3 and R5 obtained a quadratic trend, reaching the highest and lowest value at 88 and 57 days of storage (69.97 and 71.67%, respectively).

With quadratic behavior, ADF content of the whole corn plant silage harvested at the stage R3 reached a higher value at 114 days of storage (30.97%), while at R4 and R5 it reached lower values at 74 and 76 days of storage (22.01 and 23.15%, respectively). The same trend can be observed for grainless corn plant silages at stages R1, R2, R3, R4, and R5, reaching lower values at 82, 79, 85, 69, and 65 days of storage (40.10, 38.83, 41.42, 41,81, and 42.95%, respectively), while at R6, the ADF content increased linearly, 0.02% day^{-1} (Table 3).

Despite these aforementioned behaviors, the NDF and ADF levels of the whole plant were higher at stages R1 and R2, while for grainless plants, higher values were found at stages R6 and R5. It is noteworthy that, although R4 and R5 showed similar behaviors, both for whole plants and for ensiled grainless plants, stage R4 had lower NDF and ADF contents.

The ADL contents of the whole plant silage increased linearly when made in R1 (0.007% day^{-1}) and R4 (0.008% day^{-1}). Therefore, silages of grainless plants showed alterations in the ADL contents at stages R1, R2, R3, R4, and R6, all which with an increasing linear trend of 0.006, 0.006, 0.005, 0.006, and 0.003% day^{-1}, respectively (Table 3).

Regarding the maturation stages, the whole plant silage made at R4, even with a growing linear trend, had the lowest ADL content, but the same did not repeat for the grainless plant silage; however, stage R4 was close to the younger reproductive stages, which tend to have lower ADL levels due to the physiological cycle of the plant.

Crude protein values of the ensiled whole plant changed ($P<0.05$) at stages R1, R2, and R4 during the storage period (Table 4). Both with a quadratic tendency, R1 and R2 reached the highest levels at 110 and 84 days of storage (7.27 and 7.76%, respectively), while R4 reached the lowest CP content at 46 days of storage (5.23%).

The CP of the grainless plant silage changed ($P<0.05$) at stages R1, R2, R4, and R6 during the storage period. In a linear fashion, stages R1 and R2 reduced CP by 0.02 and 0.01% day^{-1}, while R6 increased by 0.009% day^{-1}, and R4 with quadratic tendency, reached the lowest CP level at 72 days of storage (5.85%).

Table 3 - Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents of whole plant and grainless plant silages prepared at different stages of corn maturity according to different storage times

	Whole plant silage							Grainless plant silage						
	Storage time (days)				Regression equation $\hat{Y}$ =	CV (%)	R^2	Storage time (days)				Regression equation $\hat{Y}$ =	CV (%)	R^2
	30	60	90	120				30	60	90	120			
	NDF (% DM)							NDF (% DM)						
R1	70.37	70.83	71.29	71.74	69.91 + 0.01D*	0.55	0.66	57.12	60.95	64.77	68.59	53.31 + 0.13D*	2.12	0.83
R2	68.41	69.75	69.83	68.66	65.81 + 0.11D – 0.0007D²*	0.92	0.59	67.29	67.41	67.17	66.57	67.11ns	1.52	0.03
R3	56.09	57.13	57.09	55.96	56.56ns	2.32	0.16	66.28	68.34	68.97	68.15	62.78 + 0.14D – 0.0008D²*	1.49	0.47
R4	51.00	50.01	49.72	50.18	50.22ns	2.30	0.19	69.61	70.27	70.57	70.51	70.24ns	1.45	0.15
R5	56.43	56.32	56.76	57.74	56.81ns	1.80	0.17	72.19	71.68	72.43	74.44	73.96 – 0.08D + 0.0007D²*	1.07	0.66
R6	57.42	57.64	57.69	57.56	57.57ns	1.64	0.02	74.13	74.86	75.04	74.69	74.68ns	0.66	0.34
	ADF (% DM)							ADF (% DM)						
R1	45.13	45.16	45.18	45.20	45.16ns	2.60	0.05	42.83	40.59	40.16	41.52	46.86 – 0.16D + 0.001D²*	3.79	0.62
R2	37.15	37.69	38.23	38.77	37.96ns	2.79	0.24	42.40	39.36	39.02	41.38	48.14 – 0.23D + 0.001D²*	2.67	0.67
R3	27.39	31.87	32.75	30.03	19.30 + 0.33D – 0.002D²*	3.83	0.78	42.92	41.73	41.43	42.04	45.01 – 0.08D + 0.0005D²*	1.22	0.58
R4	25.11	22.37	22.32	24.98	30.54 – 0.22D + 0.001D²*	3.90	0.74	43.74	41.90	42.40	45.24	47.93 – 0.17D + 0.001D²*	1.29	0.87
R5	26.60	23.59	23.45	26.19	32.50 – 0.24D + 0.001D²*	5.17	0.61	43.57	42.96	43.25	44.44	45.08 – 0.06D + 0.0005D²*	0.86	0.82
R6	26.37	26.58	26.78	26.96	26.67ns	2.22	0.15	45.04	45.75	46.47	47.18	44.33 + 0.02D*	1.54	0.59
	ADL (% DM)							ADL (% DM)						
R1	7.10	7.31	7.52	7.73	6.89 + 0.007D*	1.93	0.76	5.95	6.13	6.31	6.49	5.77 + 0.006D*	2.99	0.61
R2	6.33	5.72	5.47	5.57	5.63ns	4.38	0.13	6.14	6.35	6.55	6.75	5.94 + 0.006D*	3.20	0.59
R3	5.37	5.37	5.35	5.31	5.39ns	3.43	0.21	6.50	6.66	6.83	7.00	6.33 + 0.005D*	2.88	0.52
R4	4.52	4.76	5.00	5.24	4.28 + 0.008D*	4.84	0.61	6.60	6.78	6.96	7.14	6.42 + 0.006D*	3.94	0.40
R5	6.36	6.33	6.34	6.39	6.35ns	2.83	0.07	7.63	7.67	7.63	7.49	7.60ns	2.87	0.17
R6	6.50	6.58	6.59	6.53	6.55ns	2.53	0.03	7.48	7.58	7.68	7.78	7.38 + 0.003D*	2.14	0.34

DM - dry matter; R - scale of definition of the maturity stage of the corn plant, described by Ritchie et al. (2003); D - storage days; R^2 - coefficient of determination; CV - coefficient of variation.
* P<0.05 and ns - non-significant by Tukey's test.

Table 4 - Values of crude protein (CP) and non-fiber carbohydrates plus ether extract (NFC + EE) content of whole plant and grainless plant silages at different maturity stages of corn according to different storage times

	Whole plant silage							Grainless plant silage						
	Storage time (days)				Regression equation $\hat{Y}$ =	CV (%)	R^2	Storage time (days)				Regression equation $\hat{Y}$ =	CV (%)	R^2
	30	60	90	120				30	60	90	120			
	CP (% DM)							CP (% DM)						
R1	6.23	7.01	7.44	7.50	$5.08 + 0.04D - 0.0002D^{2*}$	4.86	0.74	8.64	7.99	7.34	6.70	$9.29 - 0.02D^*$	9.60	0.43
R2	6.87	7.58	7.74	7.37	$5.63 + 0.05D - 0.0003D^{2*}$	3.08	0.69	5.72	5.42	5.12	4.82	$6.02 - 0.01D^*$	7.00	0.48
R3	5.90	6.05	6.01	5.80	5.94^{ns}	5.94	0.12	6.31	6.57	6.78	6.93	6.64^{ns}	5.13	0.31
R4	5.29	5.27	5.62	6.33	$5.66 - 0.01D + 0.0002D^{2*}$	4.38	0.47	6.37	5.89	5.95	6.55	$7.39 - 0.04D + 0.0003D^{2*}$	8.14	0.39
R5	4.95	4.97	4.98	4.96	4.96^{ns}	4.29	0.004	5.54	5.70	5.79	5.81	5.71^{ns}	7.78	0.07
R6	4.92	5.05	5.21	5.41	5.15^{ns}	5.82	0.36	5.72	5.99	6.26	6.53	$5.45 + 0.009D^*$	6.71	0.39
	NFC + EE (% DM)							NFC + EE (% DM)						
R1	20.72	19.82	18.92	18.02	$21.62 - 0.03D^*$	3.41	0.75	27.09	26.31	25.42	24.43	25.81^{ns}	4.94	0.12
R2	21.24	19.45	19.63	21.79	$25.02 - 0.15D + 0.001D^{2*}$	3.64	0.68	23.72	23.84	24.14	24.62	24.08^{ns}	4.34	0.14
R3	35.25	34.25	34.33	35.49	34.83^{ns}	4.35	0.13	24.43	22.39	21.97	23.17	$28.08 - 0.14D + 0.0009D^{2*}$	5.12	0.46
R4	41.28	42.57	42.77	41.89	42.13^{ns}	2.84	0.22	20.26	20.26	19.89	19.17	19.89^{ns}	5.07	0.33
R5	36.23	36.64	36.51	35.84	36.30^{ns}	2.86	0.18	19.51	18.79	18.07	17.35	$20.24 - 0.02D^*$	4.11	0.56
R6	35.49	35.53	35.56	35.56	35.53^{ns}	2.23	0.0005	17.07	15.78	15.38	15.89	$19.26 - 0.08D + 0.0005D^{2*}$	4.43	0.56

DM - dry matter; R - scale of definition of the maturity stage of the corn plant, described by Ritchie et al. (2003); D - storage days; R^2 - coefficient of determination; CV - coefficient of variation.
* $P<0.05$ and ns - non-significant by Tukey's test.

When analyzing the maturation stage, the whole plant ensiled at stages R1, R2, and R3 were superior to the others, having a protein content of 6.23, 6.87 and 5.90% at 30 days of storage, while for the grainless plant, stage R1 was different from the others, presenting 8.64% protein at 30 days of storage. Considering the same storage period, the CP difference between the other stages was low, not reaching 1 percentage point.

For NFC + EE contents of whole plant silage, changes ($P<0.05$) were observed during the storage period at stages R1 and R2 (Table 4). With a decreasing linear trend, R1 reduced by 0.03% day^{-1}, while R2, having a quadratic trend, reached the lowest NFC + EE value at 72 days of storage (19.28%). The silage of grainless plants differed during the storage period at stages R3, R5, and R6. With a quadratic tendency, stages R3 and R6 reached their lowest levels, respectively, at 83 and 88 days of storage (21.92 and 15.38%, respectively), while R5 linearly decreased the NFC + EE by 0.02% day^{-1}.

Analyzing only the maturation stages, the NFC + EE content of the ensiled whole plant stood out in R3, while for the grainless plant, the highest values occurred at R1, R2, and R3.

We noted that the production of DM increased with advancing maturity stages (Table 5). When analyzing the storage days, only stages R2 and R5 had a significant effect, reducing by 14.79 and 20.66 kg, respectively, in each day of storage.

Evaluating the costs of the ton of DM, with the increase in volume produced with advancing stages, the costs were reduced, in which R4, R5, and R6 had lower costs. In terms of storage days, only stages R2 and R5 had a significant effect ($P<0.05$), increasing by 0.0004 and 0.0001 R$ ton^{-1} of DM in each day of storage.

Table 5 - Total production (ton ha^{-1}) and cost of dry matter (R$ ton^{-1}) of corn harvested at different maturity stages

	Storage time (days)				Regression equation $\hat{Y}$ =	CV (%)	R^2
	30	60	90	120			
	Dry matter production (ton ha^{-1})						
R1	6.65	6.49	6.33	6.16	6.40 ton^{ns}	5.67	0.34
R2	9.82	9.38	8.93	8.49	10.12 – 14.79D*	5.47	0.53
R3	15.31	15.06	14.98	15.05	15.10 ton^{ns}	5.65	0.02
R4	18.16	17.40	16.65	15.89	17.00 ton^{ns}	12.20	0.15
R5	22.94	22.32	21.70	21.08	23.5 – 20.66D*	3.06	0.54
R6	21.74	21.74	21.74	21.74	21.70 ton^{ns}	9.92	0.07
	Dry matter cost (R$ ton^{-1})						
R1	0.45	0.46	0.47	0.48	R$ 0.47^{ns}	6.33	0.18
R2	0.30	0.31	0.33	0.34	0.29 + 0.0004D*	5.68	0.45
R3	0.20	0.20	0.20	0.20	R$ 0.20^{ns}	5.79	0.0007
R4	0.18	0.18	0.18	0.18	R$ 0.18^{ns}	13.70	0.16
R5	0.13	0.14	0.14	0.14	0.12 + 0.0001D*	4.19	0.37
R6	0.14	0.14	0.14	0.14	R$ 0.14^{ns}	9.49	0.05

R - scale of definition of the maturity stage of the corn plant, described by Ritchie et al. (2003); D - storage days; ton - tonne; R^2 - coefficient of determination; CV - coefficient of variation.
* $P<0.05$ and ns - non-significant by Tukey's test.

4. Discussion

As in the present study, when ensiling the corn plant at different maturity stages, Vilela et al. (2008) found an increase in DM content with the advance of the stages, because the plant loses moisture as it gets older. However, when harvested with high moisture content, they provide a favorable environment for the development of undesirable bacteria and for the production of effluents, causing the DM content of the silage to rise (Macêdo et al., 2019). According to Kung Jr. et al. (2018), the corn plant at the time of harvest should have DM content between 30 and 40% for an adequate fermentation, values that are close to those found at stages R4 and R5 (Table 1).

The low pH at the first maturity stages (Table 2) suggests that it is an effect of the high concentration of soluble carbohydrates, predisposing an intense fermentation and a rapid drop in pH (Ribeiro et al., 2010; Behling Neto et al., 2017). Corroborating the results of the present study, Oliveira et al. (2013) also verified that the pH of the silage increased with advancing maturity stages.

The increasing linear trend in pH over the days of storage suggests that it is the effect of proteolysis during the storage period, which, according to Ferraretto et al. (2015), is a continuous and inevitable process; however, it is suggested that this reaction occurred in low intensity in silages evaluated in the present study, without causing any damage to fermentation, since at stages R1, R2, R3, R4, R5, and R6, silages at 120 days of storage had a pH of 3.8, 3.8, 3.9, 4.1, 4.1, and 4.1, values within the range recommended by McDonald et al. (1991), which is 3.8-4.2.

According to Tomich et al. (2004), silages with pH between 3.8 and 4.2 have their proteolytic enzymes inactive, which preserves the ensiled material. Silages with a pH greater than 4.2 are prone to excessive proteolysis and large-scale butyric fermentation (McDonald et al., 1991).

As in the present study (Table 2), Pereira (2019) evaluated corn silage stored for 45, 90, 180, and 360 days and reported a constant increase in the production of effluents during the storage period, around 0.007 L day^{-1}.

When plants are ensiled with high moisture content, they are sensitive to compaction and their cell wall is easily broken, promoting an increase in effluent production. This, in turn, transports nutrients out of the silos, which interferes with fermentation and impairs the nutritional quality of the forage, increasing the concentrations of fiber compounds (Monteiro et al., 2011; Macêdo et al., 2019).

On the other hand, harvesting at more advanced stages reduces the volume of effluent, due to an increase in the DM content and the cell walls are no longer easily broken (Rabelo et al., 2014); however, a balance point should be found, since the plant when harvested with DM contents above 40% predisposes to more accentuated losses as gases, which should also be monitored. By keeping the corn silage stored for 30 and 120 days, Junges et al. (2013) found average losses of 5.51% DM as gases, whereas Pereira (2019) found losses of 5.97%, close to the values found at R3 (5.43%) and lower than those obtained at R4 and R5, which averaged 7.39 and 11.12%, respectively, during the storage period (Table 2).

The most accentuated losses at advanced stages are due to the difficulty in expelling all O_2 from the ensiled mass, which extends the aerobic phase and predisposes the silage to secondary fermentations (Macêdo et al., 2019). Differences in losses between the stages may be due to the species of microorganism, considering that the plant DM content directly influences the species of microorganism that will develop inside the silos. According to Borreani et al. (2018), homofermentative bacteria promote small DM losses; however, the action of heterofermentative bacteria, clostridia, and yeasts can promote losses of up to 25, 51, and 49%, respectively.

The increase in NDF concentrations at stage R1 of the whole plant and grainless plant silages (Table 3) over the days of storage is due to the larger losses of soluble carbohydrates (Jacovetti et al., 2018). Nevertheless, the non-alteration in the NDF of the whole plant and grainless plant silages during the storage days in some stages is positive, since its increase has negative effects on animal intake and performance (Detmann et al., 2014).

The ADF reductions in silage of whole plant at R4 and R5 until 74 and 76 days of storage and in grainless plant silage at stages R1, R2, R3, R4, and R5 until 82, 79, 85, 69, and 65 days (Table 3) suggest the effect of acid degradation, which partially reduces hemicellulose (Der Bedrosian et al., 2012). The subsequent increase in these components suggests that it is the effect of a change of substrate inside the silo due to the continuous losses by effluents and gases (Table 2). In agreement with Evangelista et al. (2009), alteration of the available substrate inside the silos also modifies the type of microorganisms, being able to promote variations in the components of the silage during storage.

This change in substrate also modified the chemical reactions inside the silos, reactions that are not ceased after 21 days (Young et al., 2012). This behavior can justify the variations occurring in the other maturity stages.

The low NDF and ADF contents of the whole plant ensiled at R4 is due to its high participation of grains, which reached 48.81% (Table 1). According to Buso et al. (2018), the greater participation of grains in the plant structure raises starch concentrations, promoting dilutions in the concentrations of fiber compounds.

According to Silva et al. (2016), starch can assist in the fermentation process as a source of substrate for microorganisms, besides promoting strong influences on animal performance (Rossi et al., 2016). As this behavior does not occur for plants without grains, it justifies that the most advanced stages have higher levels of fiber compounds, since their deposition in the structure of plants is a physiological and continuous process (Garcez et al., 2016).

Saricicek et al. (2016) also found variations in chemical composition and nutritional value of silage stored for different periods. According to Der Bedrosian et al. (2012), these divergences in results reflect the maturity stage at which the plant was harvested and even the hybrid used.

The increase in ADL during the storage period has a greater relationship with the consumption of soluble carbohydrates during the fermentation process (Pereira, 2019). Its alterations are low due to the fact that the components of the plant cell wall are not the preferred targets of bacteria that promote fermentation in ensilage processes (Bueno et al., 2018). It is also worth noting that the low elevations in ADL over the days of storage is beneficial, as this component is the main responsible for limiting feed digestibility (Wolf et al., 1993).

The lower ADL concentrations in the whole plant silage made at R4 is the effect of its high participation of grains in the ensiled mass (Table 1). The increase in the participation of grains dilutes the concentrations of fibrous compounds, due to the increase in starch contents (Buso et al., 2018; Jacovetti et al., 2018).

The large gap in the ADL concentration between R4 and R5 is an effect of the deposition of this component in the most advanced stage, even if the concentrations of grains were close (Table 1). According to Velásquez et al. (2010) and Garcez et al. (2016), the deposition of fibrous compounds when the plant is closer to its physiological maturity occurs in greater intensity.

As this dilution effect does not occur in grainless plants, it justifies the fact that ADL has a more prominent increase during storage and the more advanced stages have higher levels of this compound in relation to the younger stages.

The increase in protein concentration in silages of whole and grainless plant at certain stages (Table 4) suggests a concentration effect, that is, the loss of soluble compounds via effluents made the protein content become higher. However, some stages showed no difference ($P>0.05$) over the storage days, a fact considered positive, as it indicates that the losses of soluble compounds and proteolysis during the storage period were not pronounced.

Nonetheless, the reductions are due to proteolysis, which hydrolyzes the peptide bonds of proteins through enzymes present in plant cells (McDonald et al., 1991). In contrast to the results found at some maturity stages in the present study, Young et al. (2012) and Ferraretto et al. (2015) evaluated different storage times of corn silage and reported that the proteolytic process is continuous.

The increase in NFC + EE contents of the silages of whole and grainless plant (Table 4), until a given moment in the storage period, suggests the effect of acid digestion of fiber compounds, as reported by Der Bedrosian et al. (2012). On the other hand, the increase in NFC + EE at the most advanced maturity stages, in particular at R4, is due to an increase in the participation of grains in the plant structure.

According to Buso et al. (2018) and Marafon et al. (2015), the increase in the participation of grains in the plant structure increases the concentrations of NFC.

In addition to changing the chemical quality of the plant, the evolution of its cycle increases dry biomass production (Pereira et al., 2011), which according to Artuzo et al. (2018), contributes to the dilution of production costs, inferences that justify the linear increase in costs in the stages that had their DM production reduced due to losses during fermentation (Table 5).

5. Conclusions

The greatest losses by effluents occurred at the first maturation stages, whereas the greatest losses by gases occurred at the more advanced stages, but both tended to increase with the days of silage storage. The dry matter content of the silage at R4 remained stable during the storage period. The neutral detergent fiber, acid detergent fiber and acid detergent lignin of the whole plant silage made at R4 were lower than at the other stages. In grain-free plant silages, these compounds at R4 did not differ from the first reproductive stages and over the storage period, R4 conferred a better-quality vegetative portion. When considering the chemical composition of the silages, the stability of production costs at stage R4 is the most suitable for making the silage, as long as it is kept in storage for approximately 70 days, during which time there are reductions in fibrous components and an increase in non-fibrous components.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M. Neumann. Formal analysis: A.M. Souza and M. Neumann. Investigation: E.R. Almeida, A.F. Matchula and F.B. Cristo. Methodology: M. Neumann. Project administration: A.M. Souza, E.R. Almeida and F.B. Cristo. Resources: M. Neumann. Supervision: A.M. Souza. Writing-original draft: A.M. Souza. Writing-review & editing: A.M. Souza, M. Neumann, L. Rampim and M.V. Faria.

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