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Microbiological quality of silage made from by-products of cassava starch extraction and viticulture

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Abstract

This study evaluated silages made with varying proportions of viticulture by-products (VC) and starch extraction from cassava (CSE). It attempted to determine the effects of these proportions on the microbial population, fermentative losses, and chemical composition. The treatments were specified as the proportions of VC in the silage (0 g/kg, 250 g/kg, 500 g/kg, 750 g/kg, and 1000 g/kg). Silages were evaluated before (0) and after 1, 3, 7, 15, 30, and 60 days of ensiling. The experimental design was completely randomized with five treatments, six storage times and four replications. The increased level of VC in the silage enhanced its dry matter content, ammonia nitrogen (NH_3-N), and buffering capacity, and reduced organic matter content. Fifteen days after ensiling, additional VC increased the concentration of soluble carbohydrates. The increased level of VC decreased the count of *Clostridium* spp. and lactic acid bacteria (LAB). The incidence of yeasts and enterobacteria was low in all treatments at all time points. Over time, losses as effluent and gases increased. Use of increasing proportions from VC in silage made with CSE increased the contents of dry matter and soluble carbohydrates and reduced the fermentative losses of the silage. The increased amount of VC also favoured pH reduction and reduced the proliferation of undesirable yeasts, while increasing the population of LAB.

Keywords: by-products, enterobacteria, lactic acid bacteria, microbial population
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Introduction

Use of agro-industrial by-products in animal feed can reduce production costs. Cassava roots are widely used as a source of starch in regions where it is grown. The by-product of cassava starch extraction (CSE) is used in feeding livestock in these regions, such as in southern Brazil (Gonçalves *et al*., 2014; Zambom *et al*., 2014; Zambom *et al*., 2015). However, because the dry matter content of cassava by-product is low (average 150 g/kg) (Jasko *et al*., 2011), its on-farm preservation and storage can be challenging.

Viticulture by-product, which is obtained from pressing grapes in wineries, is composed of bagasse and seeds (Kalli *et al*., 2018). Disposal of this by-product presents a consequential environmental impact and increases the cost of wine production (Correddu *et al*., 2015). Thus, it is essential to find alternatives for its use (Čolović *et al.*, 2019). One such alternative is in feeding livestock. It has a DM content of approximately 337 g/kg and a pH of around 4.11 (Santos *et al*., 2014). Owing to the low pH, VC inhibits the growth of undesirable microorganisms, such as bacteria of the genus *Clostridium,* and enterobacteria, and fungi and yeasts. The VC can be used as an alternative feed in regions with greater availability, reducing the effects of seasonality on forage production and production costs (Santos, 2014; Čolović *et al*., 2019).

Cassava starch extraction and VC can both be used in the fresh form for animal feeding. However, they do not maintain nutritional quality for long. Thus it is necessary to process them by drying or ensiling to conserve them for use as a feedstuff (Javorski *et al*., 2015; Fernandes *et al*., 2016; Massaro Junior *et al*., 2020). However, information on the characterization and use of VC as silage is scarce (Ke *et al*., 2015). Thus, it was hypothesized that the addition of VC to CSE as a product for silage making could reduce the

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proliferation of undesirable microorganisms, minimize fermentative losses, improve the chemical composition of silage, and reduce the time for silage stabilization. Effects of various proportions of CSE and VC in silage on the microbial population, fermentative losses, and chemical composition of silage were evaluated over a 60-day period after ensiling.

Material and Methods

The experiment was performed at the State University of Western Parana (24°33'27.5 "S and 54°02'56.2" W) located in the state of Paraná, Brazil. The CSE was obtained from industry and the VC was obtained from a winery, both from the western region of Paraná State, Brazil.

Extraction of the cassava starch used the wet processing method. Initially, cassava roots were washed, peeled and ground. Then the material was placed in tanks with agitators and sieves for starch extraction. The liquid slurry was decanted to obtain the starch (Sebrae, 2008). The by-product residue was obtained by filtration. Because fermentation occurred, this by-product had a pH of 4.05 when it was obtained from the industry. The chemical composition of the CSE was 131 g/kg DM, 975 g/kg organic matter (OM), 17.9 g/kg crude protein (CP), 8.0 g/kg ether extract (EE), 471 g/kg neutral detergent fibre (NDF), 313 g/kg acid detergent fibre (ADF), and 103 g/kg lignin.

The grapes were processed by first removing the stems, which were discarded, then pressed to break the skin and release the juice, which was subsequently fermented to transform the sugar into alcohol. This process was carried out in stainless steel tanks at a temperature between 25 ºC and 30 ºC over two to five days. Subsequently, the VC was removed. The pH of the viticulture residue was 3.45, and its chemical composition was 390 g/kg DM, 945 g/kg OM, 121 g/kg CP, 85.8 g/kg EE, 582 g/kg NDF, 510 g/kg ADF, and 139 g/kg lignin.

The materials were mixed in these proportions, namely 0% VC and 100% CSE, 25% VC and 75% CSE, 50% VC and 50% CSE, 75% VC and 25% CSE, and 100% VC and 0% CSE. The mixtures were homogenized and ensiled in silos that were made of polyvinyl chloride pipe, 10 cm in diameter and 50 cm in height, with a Bunsen-type valve that allowed gas to escape. The specific mass of the ensiled material was 891, 877, 900, 882, and 832 kg as-fed/m³ for silages with 0%, 25%, 50%, 75%, and 100% VC, respectively.

A layer of autoclaved and dried sand (0.5 kg) was placed in the bottom of each silo and covered with a layer of cotton cloth, to drain possible effluents and to avoid contact between the sand and the silage. The experimental silos were stored at room temperature under protection from sunlight until they were opened. When a silo was opened, a 5-cm layer of silage was discarded from the top and bottom and the remaining material was homogenized and sampled. The silages were evaluated before ensiling on days 0 and 1, 3, 7, 15, 30 and 60 days after ensiling. Thus, the experimental design was completely randomized with five treatments, six storage times and four replications.

Samples were pre-dried in a forced air oven at 55 °C for 72 hours and ground in a Willey mill (STAR FT 60, FORTINOX, São Paulo, Brazil) with a 1 mm sieve, and analysed for DM (method 930.15, AOAC 2000) and ash (method 942.05, AOAC 2000). Organic matter content was calculated as OM = 100 - ash (%). Dry matter recovery, and losses by gases and effluents were determined using equations described by Jobim *et al*. (2007):

$$DMR = \left(\frac{FMo\ x\ DMo}{FMs\ x\ DMs}\right) x100$$

where: DMR = dry matter recovery (%),
FMo = forage mass at the opening (kg),
DMo = DM content at the opening (%),
FMs = initial forage mass of the silage (kg), and
DMs = initial DM content of the ensiled forage (%).

$$GL = \left(\frac{SWs - PSo}{FMs\ x\ DMs}\right) x100$$

where: GL = gas losses (% of initial DM),
SWs = silo weight at ensiling (kg of wet weight),
PSo = silo weight at the opening (kg of as-fed),
FMs = initial forage mass of the silage (kg), and
DMs = initial DM content of the ensiled forage (%).

$$EP = \left(\frac{Wo - Sw}{GWef}\right) x1000$$

where: EP = effluent production (%),
Wo = weight of the set (silo + cover + wet sand + fabric) at opening (kg),
Sw = set weight set at ensiling (kg), and
GWef = green weight of ensiled forage (kg).

To determine NH_3-N, 200 g of each sample was pressed in a hydraulic press (P15 ST, BOVENAU, São José do Rio Preto, Brazil). The extract was centrifuged at 3000 revolutions per minute for 15 minutes with the supernatant being analysed by the distillation method with potassium hydroxide according to Fenner (1965).

On opening of a silo, the temperature was measured with a skewer-type thermometer. The pH was measured with a digital pH meter (Tec 2-mp Tecnal, Tecnal Scientific Equipment, Piracicaba, Brazil) by adding 100 mL of distilled water to 10 g of sample and allowing the mixture to rest for one hour before reading, according to Cherney and Cherney (2003). Room temperature was 27.0; 23.2; 21.0; 25.8; 24.5; 25.4, and 23.2 ºC at 0, 1, 3, 7, 15, 30, and 60 days of storage, respectively.

Buffering capacity was determined according to Playne and McDonald (1966). Approximately 15 g of the material was macerated before ensiling and then diluted in 250 mL of distilled water, titrated to pH 3.0 with hydrochloric acid (0.1 N), and subsequently titrated with sodium hydroxide (0.1 N) to pH 6.0.

Water-soluble carbohydrates (WSC) were determined using glucose as a standard (Dubois *et al*., 1956). Two hundred mg of the crushed sample was weighed into a 250 mL Erlenmeyer flask and 200 mL of distilled water was added. The flasks were then placed in an incubator with an orbital shaking table at 200 rotations per minute and held at room temperature for one hour to dissolve the sugars. After dissolving the sugars in water, the contents were filtered on quantitative filter paper with rapid filtration, retaining approximately 50 mL of the filtered liquid. Subsequently, a 0.5 ml aliquot was placed in a test tube, and 0.5 ml of 5% phenol and 2.5 mL of sulfuric acid were added, and this tube was immersed in a water and ice bath for 10 minutes. After cooling, slight agitation was applied and light transmission was read with a spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) at 490 ηm.

Microbial populations were determined with one homogenized sample from each silo. Culture techniques were performed according to Silva *et al*. (1997) adding sterile distilled water (225 mL) to 25 g of sample while stirring. From this solution, 1 mL was pipetted in successive dilutions of 10^{-1} to 10^{-5}, using test tubes containing 9 mL of sterile distilled water. Subsequently, from the diluted extracts, petri dishes were sown on the surface using 0.1 mL of inoculum per plate and 1 mL for plates sown in depth. To count the enterobacteria, samples were seeded in-depth on plates with violet red bile agar (VRB) and kept under incubation at 35 °C for 24 hours. For analysis of *Clostridium* spp., samples were sown on the surface in petri dishes with reinforced clostridial agar (RCA) maintained under anaerobic incubation in an oven with a CO_2 gas system at 35 °C for 24 hours. The LAB were sown on deMan, Rogosa and Sharpe (MRS) agar and incubated for 48 hours in an oven at 37 °C. After incubation, the bacteria population was counted in a Quebec colony counter (CP 608, Phoenix Luferco, Araraquara, Brazil) and transformed to $\log_{10}$ in as-fed.

Data were analysed using the PROC MIXED of SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA). The linear model was:

$$Y_{ijk} = \mu + VC_i + T_j + VC \times T_{ij} + e_{ijk}$$

where: Y_{ijk} = dependent variable,
μ = general mean,
VC_i = fixed effect of the level of VC (i = 1 to 5),
T_j = fixed effect of the storage time of the experimental silos (j = 1 to 6 for fermentative losses and 1 to 7 for other variables),
$VC \times T_{ij}$ = interaction effect, and
e_{ijk} = random residual error.

The errors were assumed to be normally distributed about mean 0 with a common variance denoted as σ_e^2. In the analysis of buffering capacity, the effects T_j and $VC \times T_{ij}$ were removed from the model. The effects of treatments were further studied using orthogonal contrasts to assess the linear and quadratic effects. A significance level of 5% was used for all analyses.

Results and Discussion

The addition of VC increased (P <0.001) the DM content of the silage linearly (Table 1) regardless of the evaluation for time (P = 0.873). There were VC by time interaction effects (P <0.001) on OM, NH_3-N, and WSC concentration. The effect of VC level was to decrease OM at a decreasing rate over time. This decrease became slightly smaller as the samples were ensiled for longer.

Except for the first day after ensiling (P = 0.273), there was a quadratic effect (P <0.001) of VC levels on the silage NH_3-N concentration. This effect was owing to an increase of NH_3-N until 75% VC, with a reduction in NH_3-N concentration in the silages composed of 100% VC. In general, there were linear (P = 0.010) and quadratic (P = 0.049) effects of VC levels on the WSC concentration. The WSC concentration typically increased following ensiling, reached a maximum, and then decreased with time. The maximum was observed somewhat later after ensiling in the silages that contained more VC.

There was a treatment and storage time interaction (P <0.001) effect on enterobacteria count, pH, and temperature of silage (Table 2). *Clostridium* spp. and LAB counts decreased linearly (P ≤ 0.016) with the increased level of VC. The VC did not affect (P = 0.569) the yeast population and VC linearly decreased (P = 0.008) silage buffer capacity.

There was an interaction between time and treatment effects (P = 0.004) on DMR, GL, and EP (Table 3). The VC decreased linearly (P <0.001) GL and increased linearly (P <0.001) DMR over time. Also, there was a quadratic effect (P <0.001) of VC levels on EP, with greater losses with 25% VC. In all storage times, there was a quadratic effect (P = 0.002) of VC levels on EP, with an increase followed by a reduction in EP concentrations to intermediate levels. In general, EP was reduced in the treatment with 100% of VC compared with other levels.

The DM content of silages increased linearly with the addition of VC, as a result of the higher DM content of VC compared with CSE. The DM content for treatment with 100% VC (404 g/kg) was similar to the 450 g/kg obtained by Molina-Alcaide *et al*. (2008). For the silage that contained only CSE (treatment 0%), DM content was on average 130 g/kg, corroborating the level presented by Javorski *et al*. (2015). In general, NH_3-N concentration was greater in silage produced from 25% CSE and 75% VC. In addition to increasing at ensiling, this mixture provided favourable conditions for higher proteolytic activity. The high concentration of NH_3-N could be related to the *Clostridium* spp. counts, which were elevated concurrently. According to Baron *et al*. (1986), microbial and plant proteases are more active during the anaerobic phase of ensiling. Decreased pH favours proteolysis (Muck, 2010), resulting in increased NH_3-N concentration as storage time increases. Also, reduced pH favoured the increase of NH_3-N concentration.

Efficient microbial fermentation in silages needs at least 30 g WSC/kg and low buffering capacity (Nussio *et al*., 2001). Lactic acid bacteria compete with facultative anaerobic microorganisms (such as enterobacteria, yeasts and *Clostridium* spp.) for WSC, which are substrates for their development, with lactic acid production and, consequently, a reduction in silage pH (Jobim *et al*., 2007). The WSC concentration in the present study fluctuated between times and treatments with the inclusion of VC in silage. However, the CSE silage showed lower levels of WSC, with a maximum content of 35.1 g/kg at day 1 of ensiling. Most of the silages in this study had the minimum WSC concentration necessary for fermentation, high LAB activity, and low pH values before ensiling, and might indicate low microbial activity after ensiling.

The ideal is that silages have low buffering capacity, facilitating rapid pH reduction, owing to the production of organic acids in the fermentation process (Siqueira *et al*., 2007). In all silages and times, pH values were within the ideal range, which varies between 3.8 and 4.2 (McDonald *et al*., 1991). This contributes to the conservation of silages by keeping a lesser population of undesirable bacteria, such as *Clostridium* and enterobacteria (Jobim *et al*., 2007).

The silage temperature exceeded 2 °C above room temperature up to three days of ensiling. This may be a consequence of oxygen present in the silo, which favours undesirable fermentations (Santos *et al*., 2006). After seven days of evaluation, silage temperature was similar to room temperature. Thus, the pH increase may have favoured the proliferation of undesirable microorganisms, such as *Clostridium* and enterobacteria, which degrade organic compounds (McDonald *et al*., 1991).

The *Clostridium* spp. genus reduced with storage time for all treatments, showing maximum values (7.61 log CFU/g) at three days in 100% VC silages. These values corroborate the work of Javorski *et al*. (2015), who, when evaluating the storage of the residue from the extraction of cassava starch, obtained a maximum point at the third day of ensiling with 7.58 log CFU/g, reducing with the storage time. The reduction of *Clostridium* spp. may be associated with a reduction in pH over the ensiling time, which is responsible for controlling the proliferation of *Clostridium* during the fermentation process (McDonald *et al*., 1991). Another

Table 1 Characteristics (g/kg of DM) of silages composed of cassava starch extraction and viticulture by-products at various times after ensiling (4 replicates)

Variables	Percent of viticulture by-product					SE	*P*-values				
	0	25	50	75	100		Treatment	Time	Interaction	Linear	Quadratic
Dry matter, g/kg	133	200	266	329	404	1.000	<0.001	0.871	0.873	<0.001	0.206
Organic matter, g/kg DM	972	962	956	950	949	0.300	<0.001	<0.001	<0.001	<0.001	<0.001
Day 0	975	965	959	956	945	0.700	<0.001	-	-	<0.001	0.539
Day 1	973	964	957	954	949	0.700	<0.001	-	-	<0.001	0.046
Day 3	970	961	951	952	955	0.700	<0.001	-	-	<0.001	<0.001
Day 7	971	963	957	941	941	0.700	<0.001	-	-	<0.001	0.419
Day 15	972	961	956	952	951	0.700	<0.001	-	-	<0.001	0.002
Day 30	974	962	955	945	950	0.700	<0.001	-	-	<0.001	<0.001
Day 60	971	962	958	950	954	0.700	<0.001	-	-	<0.001	<0.001
Ammonia nitrogen, % DM	0.096	0.108	0.112	0.120	0.091	0.001	<0.001	<0.001	<0.001	<0.001	0.003
Day 0	0.082	0.058	0.043	0.026	0.022	0.001	<0.001	-	-	<0.001	0.002
Day 1	0.097	0.067	0.054	0.056	0.044	0.001	<0.001	-	-	<0.001	0.002
Day 3	0.143	0.101	0.091	0.080	0.064	0.001	<0.001	-	-	<0.001	<0.001
Day 7	0.095	0.110	0.110	0.105	0.085	0.001	<0.001	-	-	0.001	<0.001
Day 15	0.087	0.112	0.135	0.155	0.114	0.001	<0.001	-	-	<0.001	<0.001
Day 30	0.085	0.127	0.151	0.172	0.117	0.001	<0.001	-	-	<0.001	<0.001
Day 60	0.085	0.182	0.203	0.247	0.195	0.001	<0.001	-	-	<0.001	<0.001
Water-soluble carbohydrate, g/kg DM	27.2	27.8	28.5	27.9	28.5	0.120	0.001	<0.001	<0.001	0.001	0.123
Day 0	21.5	28.0	27.4	20.7	22.1	0.319	<0.001	-	-	0.004	<0.001
Day 1	35.1	31.6	30.6	34.5	30.9	0.319	<0.001	-	-	0.011	0.049
Day 3	26.3	27.6	34.1	36.3	34.5	0.319	<0.001	-	-	<0.001	<0.001
Day 7	21.4	21.5	22.3	17.2	26.3	0.319	<0.001	-	-	0.010	<0.001
Day 15	27.5	27.5	32.6	32.8	34.0	0.319	<0.001	-	-	<0.001	0.270
Day 30	26.6	28.9	26.7	27.4	23.3	0.319	<0.001	-	-	<0.001	<0.001
Day 60	31.9	29.5	26.0	26.4	28.2	0.319	<0.001	-	-	<0.001	<0.001

Equations describing treatment effects: DM = 132.813 + 2.664VC, OM = 972.240 - 0.4399VC+0.00206VC^2, NH_3-N = 0.09417 + 0.00089VC – 0.000008VC^2, WSC = 27.560+0.09674VC

Table 2 Microbial levels, pH, temperature and buffering capacity of silages composed of cassava starch extraction and viticulture by-products at various times after ensiling (four replicates)

Variables	Percent of viticulture by-product						*P*-values				
	0	25	50	75	100	SE	Treatment	Time	Interaction	Linear	Quadratic
Clostridium spp., log CFU g^{-1}	7.00	6.84	6.85	6.61	6.63	0.057	0.189	<0.001	0.099	0.016	0.826
Lactic acid bacteria, log CFU g^{-1}	5.89	5.91	5.00	5.65	4.60	0.148	0.049	0.107	0.413	0.014	0.676
Yeast, log CFU g^{-1}	0.87	0.58	0.65	0.51	0.23	0.106	0.190	0.062	0.569	0.077	0.821
Enterobacteria, log CFU g^{-1}	1.85	1.42	1.82	1.50	0.47	0.070	<0.001	<0.001	<0.001	<0.001	0.002
Day 0	1.91	2.86	2.34	1.99	0.71	0.186	<0.001	-	-	0.011	0.011
Day 1	3.40	2.48	3.03	2.18	0.62	0.186	<0.001	-	-	<0.001	0.105
Day 3	2.57	2.70	1.54	1.99	0.00	0.186	<0.001	-	-	<0.001	0.110
Day 7	1.48	1.90	2.58	1.75	0.62	0.186	0.017	-	-	0.137	0.006
Day 15	1.73	0.00	1.83	1.13	1.04	0.186	0.008	-	-	0.836	0.651
Day 30	0.71	0.00	0.56	0.50	0.33	0.186	0.644	-	-	0.829	0.779
Day 60	1.12	0.00	0.87	0.94	0.00	0.186	0.103	-	-	0.305	0.782
pH	3.56	3.53	3.64	3.65	3.50	0.008	<0.001	<0.001	<0.001	0.999	<0.001
Day 0	4.05	3.68	3.60	3.54	3.45	0.010	<0.001	-	-	<0.001	<0.001
Day 1	3.88	3.58	3.48	3.39	3.31	0.010	<0.001	-	-	<0.001	<0.001
Day 3	3.77	3.68	3.68	3.68	3.62	0.010	<0.001	-	-	<0.001	0.461
Day 7	3.50	3.63	3.83	3.55	3.46	0.010	<0.001	-	-	0.022	<0.001
Day 15	3.53	3.53	3.82	3.74	3.63	0.010	<0.001	-	-	<0.001	<0.001
Day 30	3.17	3.33	3.58	3.93	3.64	0.010	<0.001	-	-	<0.001	<0.001
Day 60	3.05	3.27	3.46	3.73	3.41	0.010	<0.001	-	-	<0.001	<0.001
Temperature, ºC	26.0	25.7	25.9	26.0	26.1	0.016	<0.001	<0.001	<0.001	<0.001	<0.001
Day 0	29.6	28.0	28.9	28.7	29.2	0.040	<0.001	-	-	0.569	<0.001
Day 1	25.8	26.0	25.8	26.1	26.5	0.040	0.001	-	-	<0.001	0.021
Day 3	24.6	24.5	24.6	24.6	24.7	0.040	0.719	-	-	0.680	0.389
Day 7	25.9	25.9	25.9	26.2	26.2	0.040	0.024	-	-	0.032	0.459
Day 15	24.5	24.5	24.4	24.7	24.6	0.040	0.174	-	-	0.274	0.711
Day 30	27.5	27.1	27.3	27.3	27.3	0.040	0.057	-	-	0.493	0.219
Day 60	24.2	24.1	24.1	24.1	24.2	0.040	0.857	-	-	0.583	0.459
Buffering capacity, mg HCl/100 g silage	33.5	42.9	44.8	47.1	48.2	1.510	0.042	-	-	0.008	0.130

Equations describing treatment effects: clostridium = 6.9864 - 0.00401VC, lactic acid bacteria = 6.0215 - 0.00853VC, enterobacteria = 1.6777 + 0.01251VC - $0.00024VC^{2,}$ pH = 3.4139 + 0.006060VC - $0.00005VC^2$, temperature = 25.9191 - 0.00520VC + $0.000071VC^2$, buffering capacity = 37.519 + 0.1326VC

Table 3 Dry matter recovery, gas losses and effluent production from silages composed of cassava starch extraction and viticulture by-products at various times after ensiling (four replicates)

Variables	Percent of viticulture by-product					SE	*P*-values				
	0	25	50	75	100		Treatment	Time	Interaction	Linear	Quadratic
Dry matter recovery, %	94.5	95.8	96.6	98.9	99.6	0.200	<0.001	<0.001	<0.001	<0.001	0.849
D1	100.0	100.0	100.0	100	99.9	0.540	0.997	-	-	0.950	0.954
D3	96.9	98.1	97.7	99.6	100.0	0.540	0.070	-	-	0.092	0.908
D7	95.4	96.0	97.2	98.6	99.5	0.540	0.003	-	-	0.025	0.905
D15	90.5	95.5	96.3	98.7	99.4	0.540	<0.001	-	-	<0.001	0.170
D30	94.5	91.4	93.1	98.5	99.4	0.540	<0.001	-	-	0.001	0.023
D60	89.8	93.7	95.1	97.9	99.4	0.540	<0.001	-	-	<0.001	0.494
Gas losses, %	0.404	0.209	0.130	0.034	0.010	0.012	<0.001	<0.001	<0.001	<0.001	0.016
D1	<0.001	<0.001	<0.001	<0.001	0.004	0.032	0.999	-	-	0.981	0.982
D3	0.238	0.096	0.085	0.011	<0.001	0.032	0.056	-	-	0.072	0.522
D7	0.361	0.206	0.106	0.044	0.013	0.032	0.000	-	-	0.009	0.359
D15	0.686	0.225	0.140	0.041	0.014	0.032	<0.001	-	-	<0.001	0.010
D30	0.415	0.416	0.265	0.047	0.014	0.032	<0.001	-	-	0.001	0.660
D60	0.725	0.310	0.181	0.065	0.015	0.032	<0.001	-	-	<0.001	0.024
Effluent production, %	37.1	37.5	35.0	31.8	5.1	0.374	<0.001	<0.001	0.004	<0.001	<0.001
D1	32.2	33.1	23.9	18.2	2.3	0.990	<0.001	-	-	<0.001	0.002
D3	39.8	38.2	38.4	33.5	11.0	0.990	<0.001	-	-	<0.001	<0.001
D7	36.0	38.2	39.0	35.1	3.3	0.990	<0.001	-	-	<0.001	<0.001
D15	37.0	37.6	36.2	31.8	4.3	0.990	<0.001	-	-	<0.001	<0.001
D30	39.7	35.9	32.1	32.9	5.7	0.990	<0.001	-	-	<0.001	<0.001
D60	38.2	41.9	40.5	39.2	3.4	0.990	<0.001	-	-	<0.001	<0.001

DMR: dry matter recovery; GL: gas losses; EP: effluent production; equations describing treatment effects: DMR = 94.9346+0.04675VC, GL = 0.3903-0.00748VC+0.000037VC^2, EP = 36.054+0.2057VC-0.00504VC^2

factor that is correlated with the inhibition of these bacteria is the increase in osmotic pressure, that is, lower water activity and inhibition of growth of these microorganisms (Muck, 1988). Both factors are present with VC addition in CSE silages.

Values above 8.0 log CFU/g of LAB are favourable to rapid reduction of pH and improve the conservation of ensiled material (McDonald *et al*., 1991). However, in this study, the observed pH was lower than recommended for the LAB population, but this smaller population did not affect the pH reduction.

The low yeast population may be associated with the absence of oxygen for its proliferation and other microorganisms dominating the fermentation. In anaerobiosis, the yeasts ferment sugars in ethanol and CO_2. In aerobiosis, they degrade lactic acid in CO_2 and H_2O, raising the pH of the ensiled mass (Stefanie *et al*., 2000). When competing with LAB for the substrate at the beginning of the fermentation process, they can transform it into ethanol, affecting conservation and causing losses of DM and energy (Jobim & Gonçalves, 2003). Yeasts are found in greater amounts in silages with a longer aerobic initial phase (Jobim & Gonçalves, 2003). In the present study, the incidence of yeasts at various times and treatments was lower than those considered harmful to the silage quality, that is, lower than 5.0 log CFU/g (Woolford, 1990).

Enterobacteria has activities related to the pH of the ensiled material, which remained below 4.2 at all storage times. Enterobacteria can be inhibited when pH is below 4.5 (Stefanie *et al*., 2000), which explains the low proliferation of this microorganism with the addition of VC in the present study.

The increase of VC in the silages improved DMR, and it reduced with the ensiling time. Silages with the highest percentage of CSE had higher GL and EP over the ensiling time, which can be explained by the lower DM content of CSE. Treatments 25% and 50% VC had an increase in GL up to 30 days of storage, and then there was a reduction in this variable. The increase in GL up to 30 days of ensiling may have been caused by the action of heterofermentative bacteria that increased losses by gases in silages (Pacheco *et al*., 2014). Mota *et al*. (2011) evaluated the silage of the aerial part of four varieties of cassava and observed that, over the ensiled time, GL and EP increase. These losses can be reduced by ensiling feeds with a minimum DM content of 300 g/kg (McDonald *et al*., 1991).

Conclusion

The addition of VC to CSE by-product silage increased the content of dry matter and soluble carbohydrates and reduced the fermentative losses of silage. Moreover, pH values in silages that contained VC favoured their conservation and inhibited the proliferation of yeasts. However, the population of *Clostridium* spp. was high in these silages. Silages that are composed of CSE and VC mixtures can be stored up to 60 days.

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Authors' Contributions

CRS and MAZ were in charge of project design and writing of the manuscript. CRS, DG, AF, ASA and RCRT were in charge of project implementation. All co-authors participated in results, statistics and interpretation of the study.

Conflict of Interest Declaration

The authors declare no conflict of interest.

References

AOAC, 2000. Official methods of analysis. 17th ed. Association of Official Analytical Chemists, Inc., Maryland, USA.

Baron, V.S., Stevenson, K.R. & Buchanan-Smith, J.G., 1986. Proteolysis and fermentation of grain-corn ensiled at several moisture levels and under several simulated storage methods. Can. J. Anim. Sci. 66, 451–461. https://doi.org/10.4141/cjas86-047

Čolović, D., Rakita, S., Banjac, V., Đuragić, O. & Čabarkapa I., 2019. Plant food by-products as feed: Characteristics, possibilities, environmental benefits, and negative sides. Food Rev. Int. 35, 363-389. https://doi.org/10.1080/87559129.2019.1573431

Cherney, J.H. & Cherney, D.J.R., 2003. Assessing silage quality. In: D.R. Buxton., R.E. Muck & J.H. Harrison (editors). Silage science and technology. American Society of Agronomy, Madison, Wisconsin, Pp. 141-198.

Correddu, F., Nudda, A., Battacone, G., Boe, R., Francesconi, A.H.D. & Pulina G., 2015. Effects of grape seed supplementation, alone or associated with linseed, on ruminal metabolism in Sarda dairy sheep. Anim. Feed Sci. Technol. 199, 61-72. https://dx.doi.org/10.1016/j.anifeedsci.2014.11.002

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T. & Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350-356. https://doi.org/10.1021/ac60111a017

Fenner, H., 1965. Method for determining total volatile bases in rumen fluid by steam distillation. J. Dairy Sci. 48, 249-251. DOI: 10.3168/jds.S0022-0302(65)88206-6

Fernandes, T., Zambom, M.A., Castagnara, D.D., Tinini, R.C.R., Cruz, E.A., Eckstein, E.I. & Lange M.J., 2016. Nutritional assessment of waste of cassava starch extraction dried in cattle feed. Sem: Ciênc. Agrár. 37, 2653-2664. DOI: 10.5433/1679-0359.2016v37n4Supl1p2653

Gonçalves, J.A.G., Zambom, M.A., Fernandes, T., Mesquita, E.E., Schimidt, E., Javorski, C.R. & Castagnara, D.D., 2014. Chemical composition and profile of the fermentation of cassava starch by-products silage. Biosci. J. 30, 502-511. (in Portuguese, English abstract).

Jasko, A.C., Andrade, J.D., Campos, P.F.D., Padilha, L., Pauli, R.B.D., Quast, L.B. & Demiate, I.M., 2011. Physicochemical characterization of cassava bagasse in natura and after hydrolytic treatment. Rev. Bras. Tecnol. Agro. 5, 427-441. (in Portuguese, English abstract).

Javorski, C.R., Zambom, M.A., Pozza, M.S.S., Fernandes, T., Castagnara, D.D., Paladini, S. & Neres, M.A., 2015. Stoking residue from extraction of cassava starch without the use of storage technologies. Ciênc. Rural. 45, 405-411. https://doi.org/10.1590/0103-8478cr20131602

Jobim, C.C. & Gonçalves, G.D., 2003. Preserved fodder microbiology. Ruminants in ruminant production: Feed value of fodder (in Portuguese, English abstract). Jaboticabal: Funep.

Jobim, C.C., Nussio, L.G., Reis, R.A. & Schmidt, P., 2007. Methodological advances in evaluation of preserved forage quality. Rev. Bras. Zootecn. 36, 101-119. https://doi.org/10.1590/S1516-35982007001000013

Kalli, E., Lappa, I., Bouchagier, P., Tarantilis, P.A. & Skotti, E., 2018. Novel application and industrial exploitation of winery by-products. Bioresour. Bioprocess., 5, 46. https://doi.org/10.1186/s40643-018-0232-6

Ke, W.C., Yang, F.Y., Undersander, D.J. & Guo, X.S., 2015. Fermentation characteristics, aerobic stability, proteolysis and lipid composition of alfalfa silage ensiled with apple or grape pomace. Anim. Feed Sci. Technol. 202, 12-19. https://doi.org/10.1016/j.anifeedsci.2015.01.009

Massaro Junior, F.L., Bumbieris Junior, V.H., Zanin, E., Silva, L.D.F., Galbeiro, S., Pereira, E.S., Neumann, M. & Mizubuti, I.Y., 2020. Effect of storage time and use of additives on the quality of grape pomace silages. J Food Process Preserv. 00:e14373. DOI:10.1111/jfpp.14373

McDonald, P.J., Henderson, A.R. & Heron, S.J.E., 1991. The biochemistry of silage. Second edition. Chalcombe, Marlow, England. ISBN 0948617225. 340 pp.

Molina-Alcaide, E., Moumen, A. & Martín-García, I., 2008. By-products from viticulture and the wine industry: Potential as sources of nutrients for ruminants. J. Sci. Food Agric. 88, 597–604. DOI: 10.1002/jsfa.3123

Mota, A.D.S., Rocha Júnior, V.R., Souza, A.D., Reis, S.D., Tomich, T.R., Caldeira, L.A. & Costa, M.D., 2011. Fermentation profile and losses in the ensilage of different fractions of the aerial part from four cassava varieties. Rev. Bras. Zootecn. 40, 1466-1473. DOI: 10.1590/S1516-35982011000700010

Muck, R.E., 2010. Silage microbiology and its control through additives. Rev. Bras. Zootec. 39, 183–191. https://doi.org/10.1590/S1516-35982010001300021

Muck, R.E., 1988. Factors influencing silage quality and their implications for management. J. Dairy Sci. 71, 2992-3002. https://doi.org/10.3168/jds.S0022-0302(88)79897-5

Nussio, L.G., Simas, J.E.C. & Lima, M.L.M., 2001. Determination of the ideal maturity point for corn harvesting for silage. Corn for silage. FEALQ, Piracicaba. (in Portuguese, English abstract).

Pacheco, F.W., Carneiro, M.S.S., Pinto, A.P., Edvan, R.L., Arruda, P.C.L. & Carmo, A.B.R., 2014. Fermentation losses of elephant grass (*Pennisetum purpureum* Schum.) silage with increasing levels of (*Gliricidia sepium*) hay. Acta Vet. Bras. 8, 155-162.

Playne, M.J. & McDonald, P., 1966. The buffering constituents of herbage and of silage. J. Sci. Food Agric. 17, 264-268. DOI: 10.1002/JSFA.2740170609

Santos, E.M., Zanine, A.M. & Oliveira, J.S., 2006. Production of silage of grass tropical. Rev. Elect. Vet. 7, 1-16. (in Portuguese, English abstract).

Santos, N.W., Santos, G.T.D., Silva-Kazama, D.C., Grande, P.A., Pintro, P.M., De Marchi, F.E. & Petit, H.V., 2014. Production, composition and antioxidants in milk of dairy cows fed diets containing soybean oil and grape residue silage. Livest. Sci. 159, 37-45.

Sebrae, 2008. Estudo de mercado sobre a mandioca (farinha e fécula). Serviço de apoio às micro e pequenas empresas. http://atividaderural.com.br/artigos/5602f3e181880.pdf

Silva, N., Junqueira, V.C.A. & Silveira, N.F.A., 1997. Manual of microbiological food analysis methods (in Portuguese). Livraria Varela, São Paulo.

Siqueira, G.R., Reis, R.A., Schocken-Iturrino, R.P., Bernardes, T.F., Pires, A.J.V., Roth, M.D.T.P. & Roth, A.P.D.T.P., 2007. Chemical and bacterial additives association on the sugar cane ensilage. Rev. Bras. Zootecn. 36, 789-798. (in Portuguese, English abstract) https://doi.org/10.1590/S1516-35982007000400006

Stefanie, J.W.H., Elfeink, O., Driehuis, F., Gottschal, J.C. & Spoelstra, S.F., 2000. Silage fermentation process and their manipulation. In: Silage making in the tropics with particular emphasis on smallholders. Proc. FAO Electronic Conference on Tropical Silage. Rome Italy. Pages 17-30.

Woolford, M.K., 1990. The detrimental effect of air on silage. J. Appl. Bacteriol. 68, 101-116. https://doi.org/10.1111/j.1365-2672.1990.tb02554.x

Zambom, M., Fernandes, T., Soares, M.S.S.P., Castagnara, D.D., Neres, M.A, Javorski, C.R. & Cruz, E.A., 2014. Characteristics from wet residue silage of cassava starch with different levels of urea added. Arch. Zootecn. 63, 677-688. (in Portuguese, English abstract).

Zambom, M., Fernandes, T., Schmidt, E.L., Gonçalves, J.A.G, Soares, M.S.S.P., Javorski, CR. & Tinini, RCR., 2015. Silage of residue from the extraction of cassava starch in diets from lactating Holstein cows. Sem: Ciênc. Agrár. 36, 1701-1712.