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Conservation, fermentation, and nutritional quality of apple pomace silage with moisture-absorbing additives

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ABSTRACT. The objective of this study was to evaluate the use of moisture-absorbing additives for the conservation of apple pomace silage, and the effects on its fermentation and nutritional quality. The dry matter content (DMC) of apple pomace was determined using a microwave oven method, which was tested and compared to the standard oven method to facilitate calculation of the apple pomace quantities required to achieve 350 g kg^{-1} DMC in the ensiled mass. Five treatments were evaluated in experimental silos: fresh apple pomace (FAP); FAP with ground maize grain; FAP with soybean meal; FAP with wheat bran; and FAP with soybean hulls. A randomized block experimental design was used. Data were analyzed using the R statistical program, adopting a significance level of 0.05. The DMC obtained using the microwave oven method was similar to that obtained with the standard oven method (164.4 and 156.1 g kg^{-1}, respectively). The nutritional composition of the silages varied with additives; soybean meal increased crude protein content, while soybean hulls increased the fiber fraction ($p < 0.001$). The pH values were below 4.20. The addition of wheat bran resulted in 176.42 g kg^{-1} of ammoniacal nitrogen, indicating increased proteolysis. The incorporation of moisture-absorbing additives into apple pomace silage reduced gas losses by 20.1% and effluent losses by 65.9% ($p < 0.001$). Overall, the additives improved fermentation profile and reduced losses, enhancing the silage's nutritional composition.

Keywords: byproducts; dry matter; effluent losses; ruminant nutrition.

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Introduction

Sustainable increases in the productivity of agri-food systems are essential to meet global food demand. Challenges for these increases include the limited availability of agricultural land, current unsustainable use of natural resources, and the consequences of accelerating climate change (Food and Agriculture Organization [FAO], 2022). Strategies to reduce food waste and properly manage agricultural waste are critical for enhancing sustainability in agriculture. Using non-edible human food as animal feed is a promising strategy to reduce food competition and mitigate the environmental impacts of agricultural systems.

Apple (*Malus domestica* Borkh.) is one of the most cultivated and consumed tree fruits globally (Beigh et al., 2015). Global apple production exceeded 95 million Mg in 2022 (Food and Agriculture Organization Statistics [FAOSTAT], 2024). In Brazil, annual apple production exceeds 1 million Mg, with the South region accounting for 99.3% of national production (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina / Centro de Socioeconomia e Planejamento Agrícola [EPAGRI/CEPA], 2023). Approximately 25 to 30% of apple production is intended for the juice industry. The conventional juice extraction process yields 70 to 75% of the apple fresh weight as juice, with 25 to 30% remaining as apple pomace (Beigh et al., 2015). Consequently, annual apple pomace production in Brazil is estimated to exceed 60,000 Mg. Without proper management, apple pomace can pose an environmental challenge.

Recent research has reported benefits of incorporating apple pomace into ruminant diets, primarily due to its antioxidant compounds, particularly polyphenols, which enhance the oxidation-reduction potential and modulate lysosomal degradation processes in the ruminal fluid (Bartel et al., 2022). Additionally, apple pomace has been associated with reduced oxidation in lamb meat during storage (Alarcon-Rojo et al., 2019). Furthermore, improvements in ruminal fermentation, reduced methane emission, and enhanced nutrient digestibility have been reported (Gadulrab et al., 2023). However, the high moisture content of apple pomace (exceeding 700 g kg^{-1}) poses a challenge, necessitating effective conservation methods, such as ensiling. Feeding trials with apple pomace silage have shown positive results. Kara et al. (2018) evaluated silage of apple pomace mixed with pomegranate pomace (50%) and observed reduced dry matter content (DMC) losses without compromising quality or digestibility parameters. Ulger et al. (2018) reported that apple pomace DMC should be increased to improve silage quality when mixed with maize plants, beet pulp, or pumpkin pulp.

DMC is a critical factor for producing high-quality silage, with optimal values ranging from 250.0 to 350.0 g kg^{-1} (Kung Jr. et al., 2018). Apple pomace, like other agroindustrial residues, exhibits significant variability in moisture content. Therefore, determining DMC is the initial step in ensiling this residue. This procedure is routinely performed in animal nutrition laboratories using forced-air ovens at 55°C for 72 hours. However, this method is impractical for assessments at rural properties, making the use of a microwave oven a viable alternative for determining DMC. Protocols commonly used for drying materials in microwave ovens, including those for determining exposure time and power, are primarily designed for forages. Thus, a specific protocol for apple pomace is necessary.

Increasing the DMC of apple pomace is essential for ensiling, as contents below 250.0 g kg^{-1} create favorable environments for the proliferation and development of undesirable bacteria during the fermentation process, leading to nutrient losses through effluent production. These losses are generally substantial and adversely affect silage quality (Borreani et al., 2017). Ensiling by-products with other feedstuffs is a strategy to overcome many of these limitations, producing stable and nutritionally balanced silages without effluent losses (Dentinho et al., 2023). However, the most suitable ingredients for ensiling apple pomace remain undocumented.

In this context, the hypothesis of this stdy was that incorporating additional ingredients increases apple pomace DMC, thereby improving silage fermentation and reducing losses through gasses and effluents. Therefore, the objective of this study was to evaluate the use of moisture-absorbing additives for the conservation of apple pomace silage and their effects on fermentation and nutritional quality.

Material and methods

The experiment was conducted at the Experimental Station of the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), in Lages, Santa Catarina, Brazil (27°48'29''S and 50°19'45''W). Fresh apple pomace (FAP) was obtained in six batches from a local juice extraction processing industry, constituting six experimental blocks. Three samples from each batch were collected and dried to determine moisture content. A protocol for determining the moisture content of apple pomace using a microwave oven was developed, and the results were compared with those obtained from the standard method, which involves drying in a forced-air oven at 55°C for 72 hours.

The microwave oven method was tested at 80% power, with a glass of water placed inside the appliance to prevent sample burning. A 100 g sample of FAP was placed in the microwave for two minutes, then removed, allowed to cool, and weighed. This procedure was repeated five times, totaling ten minutes of drying. Subsequently, the exposure time was reduced to one minute, repeated five times, totaling five minutes. The exposure time was then reduced to 30 seconds until constant weight was obtained in three consecutive weighings. The resulting weight (g) represented the sample's dry matter content (DMC).

This protocol was established after several preliminary tests with different exposure time combinations to avoid sample burning, as FAP combusts readily with longer microwave exposure times. The data did not meet normality assumptions, necessitating the use of the non-parametric Mann–Whitney U test for mean comparison, performed using R version 4.2.0 (R Core Team, 2022). DMC of apple pomace was similar for both drying methods (standard error = 0.380; p-value = 0.159), with 156.1 g kg^{-1} for the standard oven method and 164.4 g kg^{-1} for the microwave oven method.

The conservation of apple pomace silage was evaluated using five apple pomace samples collected at 14-day intervals. DMC was immediately estimated using the microwave oven method. The amount of ingredient to be added to achieve a DMC of 350.0 g kg^{-1} in the ensiled mass was calculated, ranging between 23.5 and 28.3%. Five experimental treatments were evaluated: FAP alone; FAP with ground maize grain; FAP with soybean meal; FAP with wheat bran; and FAP with soybean hulls.

Experimental polyvinyl chloride (PVC) silos, 50 cm in length and 10 cm in diameter, with known weights, were used. The bottom of each silo contained 700 g dry sand, separated from the silage mixture by nonwoven fabric to quantify effluent production. After thorough homogenization of FAP with the ingredients, the mixture was placed in the silos and compacted using a wooden plunger. After filling, the silos were sealed with PVC caps fitted with Bunsen-type valves, weighed, and stored at room temperature in a ventilated, dry, covered location. After 45 days of ensiling, all the experimental silos were weighed and opened. Silages were manually removed from each experimental silo and homogenized. Samples were collected upon opening the silos and stored for subsequent analysis of fermentation profile and chemical composition.

Silage dry matter losses through gases and effluents were quantified by weight difference, according to the equations described by Zanine et al. (2010):

$$GL = \frac{(WFc - WFo)}{(WMc \times DMc)} \times 10000 \quad (1)$$

where GL = gas losses (% of dry matter), WFc = weight of the filled silo at sealing (kg), WFo = weight of the filled silo at opening (kg), WMc = weight of the ensiled matter at sealing (kg), and DMc = dry matter content at sealing (%).

$$EL = \frac{(WESo - WE) - (WESc - WE)}{WMc} \times 1000 \quad (2)$$

where EL = effluent losses (kg Mg^{-1} fresh matter), WEo = weight of the empty silo plus sand at opening (kg), WE = weight of the empty silo (kg), WEc = weight of the empty silo plus sand at sealing (kg), and WMc = weight of the ensiled matter at sealing (kg).

Silage pH and total titratable acidity were determined using the method described by Silva and Queiroz (2006). A fresh silage sample of 9 g was diluted in 60 mL of distilled water, allowed to rest for 30 minutes, and then the pH was measured using a pH meter. Total titratable acidity was determined by titration with 0.1N sodium hydroxide (NaOH) using the following equation:

$$TTA = \frac{(V \times N \times f \times 100)}{Vs} \quad (3)$$

where TTA = total titratable acidity (mEq 100 g^{-1}), V = NaOH volume used in titration (mL), N = normality of the acid, f = correction factor (1.06), and Vs = sample volume (mL).

Ammoniacal nitrogen (NH_3-N), expressed as g kg^{-1} of total nitrogen, was analyzed in 25 g silage samples by adding 200 mL of a 0.2 N sulfuric acid (H_2SO_4) solution, allowing the mixture to rest for 48 hours, and then filtering it through a Whatman 54 filter. The filtrate was distilled with 2 N potassium hydroxide (KOH) in a micro-Kjeldahl apparatus and then titrated with 0.1 N hydrochloric acid (HCl), following the procedure outlined by Bolsen et al. (1992).

The silage ingredients (FAP, ground maize grain, soybean meal, wheat bran, and soybean hulls) were analyzed for dry matter (method 934.01), ash (method 930.05), and crude protein (method 920.87) contents, as described by the Association of Official Analytical Chemists (AOAC, 2012). Neutral detergent fiber and acid detergent fiber were determined using a fiber analyzer with filter bags, as described by Detmann et al. (2012), and in vitro organic matter digestibility was assessed as described by Tilley and Terry (1963). The chemical-bromatological composition of the silage ingredients before ensiling is presented in Table 1.

Table 1. Chemical composition of fresh apple pomace (FAP), ground maize grain (MG), soybean meal (SM), wheat bran (WB), and soybean hulls (SH).

Parameter (g kg^{-1})	FAP	MG	SM	WB	SH
Dry matter	126.4	879.1	902.7	891.1	914.1
Ash	19.0	12.5	61.7	60.4	114.2
Crude protein	57.5	116.3	529.0	192.1	49.1
Neutral detergent fiber	303.7	122.2	90.8	423.0	793.7
Acid detergent fiber	230.1	18.0	40.3	129.0	571.7
In vitro organic matter digestibility	744.4	913.9	933.3	655.4	223.3

Silage microbiological analysis was conducted by diluting 10 g samples in 90 mL of a 0.8% NaCl saline solution to obtain the first serial dilution. The diluted extracts (dilutions ranging from 10^{-1} to 10^{-6}) were inoculated onto specific media for each microorganism studied. Lactic Acid Bacteria counts were determined using Man, Rogosa, and Sharpe Agar (Kasvi), incubated at 28 ± 1°C for 72 hours, as described by Jonsson (1991). Potato Dextrose Agar (PDA) with 0.04% chloramphenicol was used for yeast count, incubated at 30 ±

1°C for 72 hours, as described by Tengerdy et al. (1991). Reinforced Clostridial Agar was used for Clostridium counts, with anaerobic incubation at 35 ± 1°C for 48 hours, as described by Tosi et al. (1982). Colony numbers were expressed as colony-forming units and transformed into log^{10} values.

The experiments were conducted in a randomized complete block design with five treatments and ten replicates (experimental units), using sampling dates as blocks. Data were subjected to the Shapiro-Wilk test for normality of residuals, the Bartlett test for variance homogeneity, and analysis of variance (ANOVA). Significant means by the F-test ($p < 0.05$) were compared using Tukey's test. All values were reported as means with pooled standard errors of the means. All statistical analyses were performed using R version 4.2.0 (R Core Team, 2022).

Results and discussion

Several factors influence the moisture content of apple pomace, including apple variety, cultivation practices, fruit maturity, post-harvest handling, and type of industrial processing. The scientific literature reports a wide range of dry matter content (DMC) for fresh apple pomace (FAP), from 149.4 g kg^{-1} (Ribeiro Filho et al., 2012) to 360.0 g kg^{-1} (Halmemies-Beauchet-Filleau et al., 2018). According to Kung Jr. et al. (2018), the recommended DMC for silages ranges from 250.0 and 350.0 g kg^{-1}. Therefore, measuring DMC and calculating the amount of ingredient to be added to achieve a target DMC of 250 and 350 g kg^{-1} in the final mixture is recommended due to this wide DMC variability.

The chemical-bromatological composition of silages varied depending on the ingredient added (Table 2). The addition of ingredients effectively increased silage DMC ($p < 0.001$). FAP silage had a DMC of 105.8 g kg^{-1}, while the other treatments ranged from 276.6 to 304.0 g kg^{-1}, highlighting the hygroscopic effect of the ingredients. Ulger et al. (2018) combined apple pomace with maize silage (50:50), achieving a DMC of 213.2 g kg^{-1}, and concluded that increasing apple pomace DMC is necessary to produce high-quality silage.

Table 2. Chemical-bromatological composition of silages composed of fresh apple pomace (FAP), FAP with ground maize grain (FAP MG), FAP with soybean meal (FAP SM), FAP with wheat bran (FAP WB), and FAP with soybean hulls (FAP SH).

Parameter (g kg^{-1})	FAP	FAP MG	FAP SM	FAP WB	FAP SH	SEM	*p*-value
DMC	175.8c	304.6a	278.6b	276.6b	304.3a	11.38	< 0.001
Ash	43.2c	17.4d	61.3b	51.7b	115.6a	5.11	< 0.001
CP	69.2d	91.1c	433.3a	175.5b	57.1d	20.16	< 0.001
NDF	463.1b	194.1d	198.7d	397.6c	731.4a	28.57	< 0.001
ADF	370.8b	93.7e	111.3d	180.2c	548.4a	24.92	< 0.001
IVOMD	639.2c	744.6b	852.1a	665.0c	318.4d	26.18	< 0.001

SEM = Standard error of the mean; DMC = dry matter content; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; IVOMD = In vitro organic matter digestibility. Means followed by different letters in the rows significantly differ by the Tukey's test ($p < 0.05$).

Crude protein content was higher in silages with soybean meal (433.3 g kg^{-1}; $p < 0.001$) compared to other treatments, as expected, while FAP alone and FAP with soybean hulls silages resulted in lower mean crude protein contents, averaging 63.1 g kg^{-1}. Adding urea is an alternative to increase crude protein content of silages. Pirmohammadi et al. (2006) evaluated apple pomace silage with wheat straw and reported a 12.5% increase in crude protein content when adding urea (5 g kg^{-1}, fresh weight basis). The fiber fraction was higher in silages with soybean hulls, with 731.4 g kg^{-1} for neutral detergent fiber and 548.4 g kg^{-1} acid detergent fiber. Consequently, *in vitro* organic matter digestibility was reduced to 318.4 g kg^{-1} in these silages. The soybean hulls used were of poor quality (Table 1), which adversely influenced the nutritional quality of the silage. The choice of ingredient to be mixed should consider both economic and nutritional factors. Using residues for moisture absorption reduces effluent losses and improves the fermentation profile of silages, but often at the expense of nutritional composition and nutrient digestibility.

Ulger et al. (2018) reported in vitro organic matter digestibility of 623.2 g kg^{-1} for apple pomace silage, which aligns with the results of the present study. These results demonstrated the efficacy of incorporating soybean meal or ground maize grain into apple pomace silage to enhance its nutritional quality. Adding soybean meal or ground maize grain significantly improved the nutritional quality of FAP silages, as evidenced by the *in vitro* organic matter digestibility values of 852.1 and 744.6 g kg^{-1}, respectively, compared to 639.2 g kg^{-1} for the silage FAP alone ($p < 0.001$). These findings suggest that tailored nutritional strategies can be developed to meet herd nutritional requirements based on nutrients provided by pastures. For example, soybean meal can address protein deficiencies, while ground maize grain can support finishing purposes.

The pH of silages with added ingredients fell within the recommended range (>3.80 and <4.20) established by McDonald et al. (1991) (Table 3). However, the pH of FAP silage alone was below this range (3.40), consistent with previous studies by Kara et al. (2018) and Islam et al. (2014), who reported pH values of 3.57 and 3.60, respectively, for FAP silages. Silage pH approaching 4.0 enhances preservation by inhibiting the activity of *Clostridium* bacteria, which are associated with butyric acid production and silage deterioration (Kung Jr. et al., 2018). The addition of soybean meal, wheat bran, or ground maize grain increased total titratable acidity compared with FAP silage alone ($p < 0.001$). According to Silva and Queiroz (2006), total titratable acidity is strongly correlated with lactic acid content, which is not always accurately reflected by pH analysis alone. Thus, these treatments provided a superior fermentation profile with increased lactic acid production.

Ammoniacal nitrogen (NH_3-N) content is a critical factor for assessing fermentation processes, indicating the extent of proteolysis. NH_3-N values below 100.0 g kg^{-1} of total nitrogen indicate silage with satisfactory fermentation profiles (Kung Jr. et al., 2018). Only the treatment with wheat bran differed from the others ($p < 0.001$), resulting in NH_3-N contents of 176.4 g kg^{-1} of total nitrogen. According to Bernardez et al. (2005), high ammonia production may result from a low quantity of soluble carbohydrates in the substrate, leading lactic acid bacteria to utilize amino acids as an energy source for growth and metabolism. Wheat bran has a low soluble carbohydrate content (60 g kg^{-1} DMC), which was insufficient to induce a rapid pH drop and inhibit protein degradation (Nogueira et al., 2019).

Table 3. Physicochemical and microbiological parameters of silages composed of fresh apple pomace (FAP), FAP with ground maize grain (FAP MG), FAP with soybean meal (FAP SM), FAP with wheat bran (FAP WB), and FAP with soybean hulls (FAP SH).

Parameter	Unit	FAP	FAP MG	FAP SM	FAP WB	FAP SH	SEM	*p*-value
pH	-	3.4e	3.6d	4.2a	4.0b	3.8c	0.04	< 0.001
NH_3-N	g kg^{-1} TN	71.7b	85.2b	41.1b	176.4a	48.0b	0.95	< 0.001
TTA	mEq 100 g^{-1}	2.6d	2.9c	4.2a	3.6b	2.7cd	0.10	< 0.001
GL	g kg^{-1} DMC	199.6a	157.0c	159.7bc	164.4b	157.0c	0.28	< 0.001
EL	kg Mg^{-1}	109.1a	90.4b	7.7d	22.8cd	28.2c	6.41	< 0.001
LAB	log CFU g^{-1}	7.0	6.9	7.6	7.5	7.4	0.13	0.419
CL	log CFU g^{-1}	7.4	7.5	7.8	7.7	7.6	0.11	0.435
Yeasts	log CFU g^{-1}	6.0	5.0	5.8	5.6	4.9	0.23	0.309

SEM – Standard error of the mean; NH3-N = ammoniacal nitrogen; TTA = total titratable acidity; GL = gas loss; EL = effluent loss; LAB = lactic acid bacteria; CL = Clostridium; CFU = colony-forming units. Means followed by different letters in the rows significantly differ by the Tukey's test ($p < 0.05$).

The hypothesis of this study was confirmed by the reduction in gas and effluent losses resulting from the addition of ingredients, while FAP silage alone exhibited the highest losses ($p < 0.001$). Gas losses were lowest for silages with ground maize grain or soybean hulls, averaging 157.0 g kg^{-1} DMC, followed by soybean meal (159.7 g kg^{-1}) and wheat bran (164.4 g kg^{-1}), whereas FAP silage alone had gas losses of 199.6 g kg^{-1}. Gas losses result from the production of carbon dioxide, hydrogen, and ethanol during lactate or hexose fermentation (McDonald et al., 1991), reducing silage quality due to the loss of highly digestible compounds, including soluble carbohydrates, organic acids, minerals, and nitrogen compounds (Grizotto et al., 2020). Grizotto et al. (2020) reported a 67% reduction in gas losses when incorporating 20% pelleted citrus pulp into orange peel silage.

High effluent production in silages leads to nutrient losses through leaching, compromises the nutritional value, adversely affects soil microbiota, and increases greenhouse gas emissions, including nitrous oxide (Araújo et al., 2020). Silage with soybean meal reduced effluent production by 92.9% compared to FAP silage alone and by 17.7% compared to silage with ground maize grain. Ferreira et al. (2023) reported significant reductions in gas and effluent losses by adding by-products (meal or cake) from babassu (*Attalea speciosa*) processing to Guinea grass (*Megathyrsus maximus*) silages, emphasizing the importance of adjusting the DMC of the ensiled mass. The addition of ingredients to FAP increased the DMC of silages by an average of 65.9%, which was the primary factor in reducing effluent losses.

Although silages exhibited different fermentation parameters, variations in microbial populations among treatments were not significant ($p > 0.05$; Table 3). According to McDonald et al. (1991), a minimum of 8.0 log CFU g^{-1} DMC of LAB is required for a rapid pH reduction; in this study, the average observed was 7.3 log CFU g^{-1} DMC. Given that LAB populations varied by incubation day (Bernardes et al., 2005) and that pH values remained below 4.20, the growth of these bacteria was adequate across all treatments. The average yeast count was 5.46 log CFU g^{-1} ($P=0.309$). Silages with yeast counts exceeding 5.0 log CFU g^{-1} are highly susceptible to spoilage (Ávila and Carvalho, 2020; Borreani et al., 2017), predisposing the evaluated silages to rapid surface deterioration upon silo opening. In this context, silo size should be determined based on the daily silage required. Low *Clostridium* species growth is desirable, as these are the main silage spoilage microorganisms (Mota et al., 2011). *Clostridium* species were abundant (7.62 log CFU g^{-1} DMC, on average) after silo opening. Both pathogenic and non-pathogenic *Clostridium* strains are identified in silages (Kobayashi et al., 2017; Driehuis et al., 2018), with proteolytic variants predominating. Their presence often indicates contamination of the source material (Ávila and Carvalho, 2020), necessitating further investigation into specific *Clostridium* species in apple pomace and their potential implications for the safety of this silage byproduct.

Conclusion

The conservation of apple pomace as silage is feasible, and the addition of moisture-absorbing ingredients results in reduced gas and effluent losses and a satisfactory fermentation profile. The nutritional composition of silage varies depending on the ingredient used, enabling to choose ingredients according to the nutritional needs of the herd.

Data availability

We inform you that the data used in the research were deposited on the OSF platform and can be accessed through the link https://osf.io/vstm5/

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