

Effects of Moisture Content and Additives on the Fermentation Quality and Degradation of Glycoalkaloids in Potato (*Solanum tuberosum*) Vine Silage in Tibet

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Abstract: The objectives of this research were to evaluate the effects of raw material moisture content and additives on the fermentation quality and *degradation of* glycoalkaloids in potato vine silage and to explore new approaches for feedstuff preservation with the aim of providing a source of sustainable livestock feed. Potato vine was partially wilted to three different target moisture contents [approx. 75% (M1), 65% (M2), and 55% (M3)] and treated with (1) formic acid [1.5% fresh weight (FW), FA]; (2) pre-fermented juices (5.0 mL kg⁻¹ FW, PFJ); (3) corn flour (100 g kg⁻¹ DM, CF); (4) potato pulp (30% FW, PP); and (5) no additives (control). After 45 days of ensiling with polyethylene (100 mL), the fermentation quality, chemical composition, and concentration of glycoalkaloids were determined. The results showed that silage quality and glycoalkaloid concentration were significantly influenced by moisture content and additives ($P < 0.05$). Lactic acid (LA), pH, acid detergent fiber (ADF), and neutral detergent fiber (NDF) increased slightly with decreasing moisture content; in contrast, the concentration of LA/AA declined. LA content was highest and pH and acetic acid (AA) were lowest at M1 compared with M2 and M3. Little to no butyric acid (BA) was detected in the presence of additives. The FA-treated silage exhibited a significantly reduced pH value and ammonia-N/total-N (NH₃-N/TN) content ($P < 0.05$) and an increased concentration of LA and water-soluble carbohydrates (WSCs). PP-treated silage provided sufficient fermentation substrate, and the DM and WSC contents increased significantly ($P < 0.05$) compared with the PFJ and CF treatments. Supplementation with PFJ resulted in the pH of the ensiled forage stabilizing at approximately 4.40. With the addition of CF, the LA:AA ratios of the different moisture content treatments were 2.42, 2.15, and 1.75, respectively, which were significantly lower than 3:1 in the other treatments at all moisture contents. The potato glycoalkaloid content of the PV silage increased with decreasing moisture level. Glycoalkaloid concentration was significantly reduced to 0.55, 4.57, and 7.73 100 mg g⁻¹, respectively ($P < 0.05$), in the different moisture treatments by the addition of FA. In conclusion, the best quality PV silage was produced at 75% moisture content with the addition of FA. Additive ensiling thus constitutes an effective approach for potato vine preservation.

Keywords: Additives, Fermentation Quality, Lactic Acid Bacteria, Moisture, Potato Vine Silage, Tibet

1. Introduction

The Qinghai-Tibet Plateau is a globally important

ecological region wherein animal husbandry constitutes the primary industry. Livestock feed shortages are increasingly becoming one of the principal challenges in livestock production and development worldwide. It is therefore

imperative that the use of potential nonconventional alternatives, such as agricultural industry by-products or straw, is explored for application in animal feed.

Potato (*Solanum tuberosum* L.) is one of the top four-most cultivated food crops of the Tibetan Plateau, with an annual cultivation area of approximately 16 thousand hectares [1] and an annual potato vine yield of 240 thousand tons [2]. Potato vine typically contains 80–260 g crude protein (CP) kg⁻¹ dry matter (DM), 230–453 g neutral detergent fiber (NDF) kg⁻¹ DM, and the intermediate solanesol, which is used in ubiquinone drug synthesis [3, 4]. This dry matter and fiber could potentially constitute valuable fodder for ruminants. However, fresh potato vine is not a suitable feed for ruminants because of its poor palatability and high glycoalkaloid content [5, 6], and it is thus considered as waste [7].

Ensiling is regarded as an efficient way to maintain the nutritive value and improve the palatability of forage. Recent studies have focused on the use of conventional alternatives, such as alfalfa (*Medicago sativa* L.) [8], corn (*Zea mays* L.) [9], and oats (*Avena sativa* L.) [10]. The optimization of moisture content and additives has also been addressed [11, 12]. The initial moisture content greatly influences silage characteristics by facilitating the fermentation process [13], and the addition of formic acid (FA) to the silage effectively inhibits microbial activity [14–16]. Zhu *et al.* [16] showed that pre-fermented juices could significantly increase dry matter and water-soluble carbohydrate concentration, as well as decreased protein degradation ($P < 0.05$) and improved the fermentation quality of alfalfa silage. Potato pulp is one waste product that is not efficiently utilized and has potential to be used as a substrate for improving ensiling quality due to its high vitamin and pectin contents [17]. In addition, the polyphenol oxidase and caffeic acid present in potato pulp are inhibitors of protein degradation [18]. Zhang *et al.* [19]

reported that the combination of potato pulp and rice straw resulted in an increase of crude protein (CP), water-soluble carbohydrate (WSCs) and lactic acid ($P < 0.05$), as well as decreased pH and neutral detergent fiber (ADF) contents in rice straw. Substantial efforts are currently being made to develop new and improved ensilage treatments, but few studies have focused on the use of agricultural by-products. Currently, most studies on potato vine have reported on glycoalkaloids chemical composition and have explored the ensiling process [20, 4], and glycoalkaloid toxicity in small ruminants [21, 22], whereas limited studies on evaluating the degradation of glycoalkaloids, and the quantitative degradation of potato glycoalkaloids during ensiling [7, 23].

The objectives of the present study were to investigate the effects of raw material *moisture content and additives on the fermentation quality and degradation of potato glycoalkaloids in potato vine silage and determine the optimal moisture content and additives conditions*, our aim was to explore the efficient development and utilization of the agricultural by-product potato vine as a source of sustainable livestock feed.

2. Materials and Methods

2.1. Forage and Ensiling

2.1.1. Ensilage Preparation Procedure

Potato was harvested at the mature stage. Fresh potato vine was obtained from the experimental field of the Institute of Vegetable, Tibet Academy of Agriculture and Animal Husbandry Science. The potato vines were cut at approximately 8 to 10 cm above the ground using a sickle, and then chopped into 2–3 cm segments. Before ensiling, the chemical composition of the potato vine sample was measured as shown in Table 1.

Table 1. Chemical components of potato vine.

Item	WSC (% DM)	CP (% DM)	NDF (% DM)	ADF (% DM)	BC (mE kg ⁻¹ DM)	Potato glycoalkaloids (mg 100g ⁻¹ DM)
Potato vines	3.68±0.17	18.49±0.65	35.33±1.03	29.60±0.55	694.11±6.13	107.06±4.25

Note: Water-soluble carbohydrate, WSC; crude protein, CP; neutral detergent fiber, NDF; acid detergent fiber, ADF; buffering capacity, BC; dry matter, DM.

Corn meal (CM) was purchased from the supermarket. Potato pulp (PP) was kindly provided by the Hengxin starch factory in Dingxi (Gansu, China) (175 g pectin kg⁻¹, DM). Analytical grade formic acid (FA) (85%) was obtained from Beijing Chemical Works (Beijing, China).

The preparation of the pre-fermented juices (PFJ) followed the method of Song *et al.* [24]. Briefly, 200 g of the pre-ensiling sample with homogenized by placing in a blender with 1000 mL deionized water and blending for 30 min at room temperature. Following this, the mixture was filtered through two layers of pledget. After the addition of 2% sucrose, the filtrate was shaken and quickly placed into a brown glass bottle and fermented for 48 h at 30°C in an incubator.

2.1.2 Ensiling

The chopped potato vine was partially wilted to three

different target moisture contents [approx. 75% (M1), 65% (M2), and 55% (M3)], and the moisture contents were continuously measured using an inductive moisture meter and microwave oven. They were divided into different equal parts and mixed in the following treatments: (1) 1.5% FA (A1) on a FW basis; (2) 1.5% PFJ (A2) on a FW basis; (3) 100 g·kg⁻¹ CF (A3) on a DW basis; (4) 30% PP (A4) on a FW basis; and (5) no additives (control). The experimental design is indicated in Table 2. After mixing thoroughly, the ensiling materials were packed into the silos (100 mL polyethylene plastic vials) and sealed with a screw top and Parafilm. Each silo was prepared in five replicates. All silos were stored indoors for 45 d at an ambient temperature of approximately 22 ± 2°C.

Table 2. Experimental design.

Moisture content (%)	Additives				
	Formic acid (A1)	Pre-fermented juices (A2)	Corn flour (A3)	Potato pulp (A4)	Control (A5)
75 (M1)	M1 A1	M1 A2	M1 A3	M1 A4	M1 Control
65 (M2)	M2 A1	M2 A2	M2 A3	M2 A4	M2 Control
55 (M3)	M3 A1	M3 A2	M3 A3	M3 A4	M3 Control

2.2. Chemical Composition Analyses

2.2.1. Sensory Evaluation

After opening the silos, the color, odor, and texture were used to evaluate the quality of the silage.

2.2.2. Fermentation Metabolite Analysis

Water extracts of the silages were prepared by placing 20 g pulverized feedstuff and 180 mL deionized water into an Erlenmeyer flask, which was then kept in a refrigerator at 4°C for 24 h. The homogenate was then filtered through four layers of pledget and one layer of qualitative filter paper. Water extracts were stored at -20°C for the determination of pH, buffering capacity (BC), NH³-N, LA, and volatile fatty acid (VFA) contents. The pH value was measured directly using a pH meter (PHS-3C, Ray Magnetic Instrument Factory, Shanghai, China). Buffering capacity was determined using the hydrochloric acid-sodium hydroxide method of Playne and McDonald [25]. The concentration of NH³-N was determined according to a colorimetric method [26]. The thawed extracts were centrifuged for 15 min at 10,000 rpm and filtered through a dialyzer (pore size of 0.22 μm) for the determination of LA and VFAs by high-performance liquid chromatography (HPLC) on an Agilent 1100 series HPLC [Agilent Technologies, Santa Clara, CA, USA; HP1100LC model, KC-811 column (15 cm×4.6 mm×5 μm), ultraviolet detector G1314B]. The mobile phase was 0.01 mol L⁻¹ NaH₂PO₃ (disodium hydrogen phosphite) solution, and the pH was approximately 2.32; the column temperature was 35°C; the injection volume was 10 μL; the flow rate was 1.0 mol min⁻¹; and the measured wavelength was 210 nm.

2.2.3. Pre-ensiling and Silage Analysis

The pre-ensiled materials and ensiled samples were dried at 65°C for 48 h for DM calculation according to the procedure of Yang [27] and then passed through a 1 mm screen and stored at 4°C until further analysis. The water-soluble carbohydrate (WSC) concentration was determined colorimetrically after reaction with anthrone reagent [28]. The methods of Van Soest et al.[29] were used for the determination of NDF and ADF. The CPs were analyzed using a FOSS analyzer (FOSS Electric, Denmark) following the Association of Official Analytical Chemists (AOAC) procedure for protein determination [30].

2.3. Potato Glycoalkaloid Analysis

α-Solanine standards (purity ≥ 99.8%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standard pure solanine (0.0500 g) accurately weighed and added to a 50 mL

volumetric flask with 1% ammonium hydroxide solution, which constituted the standard solution of solanine. We then pipetted 0.5, 1.0, 1.5, 2.0, and 2.5mL of the standard solution into the 5 mL volumetric flask, and the test solutions were measured at 530 nm using an ultraviolet spectrophotometer [31]. The standard curve of pure solanine was $y=0.6767x+0.0054$ ($R^2=0.9863$).

Dry samples of pure potato vines or silages (20 g) were mixed with acidified alcohol (anhydrous ethanol: acetic acid = 100:30, v/v) and transferred into a 500 mL round-bottom flask, agitated with a magnetic stirrer for 15 min [31], and then filtered. The filtrate and samples were heated in a water bath at 55–65°C for 16 h in a Soxhlet extractor, following which the solvent was evaporated off using a rotary evaporator. All the samples were dissolved and washed with 5% sulfuric acid solution and then filtered through two layers filter paper again. Upon cooling, ammonium hydroxide was added until the pH of the filtrate was within the range of 10–11. The filtrate was then centrifuged (4°C) at 5000 rpm for 15 min and the precipitates cleaned with 1% ammonium hydroxide solution. After re-centrifugation, the dried precipitate consisted of crude glycoalkaloids. The glycoalkaloids were dissolved in 1% sulfuric acid solution and combined with 10 mL water in a volumetric flask. From this latter solution, duplicate aliquots (2 mL) were mixed with 5 mL of 98% sulfuric acid, and after 3 min, 2.5 mL of 1% formaldehyde was added. After standing for 90 min, the absorbance of the test solutions was determined at 530 nm using an ultraviolet spectrophotometer. The glycoalkaloid level was recorded as solanine equivalents (mg 100g⁻¹ DW) by reference to the standard curve of pure solanine [32].

2.4. Statistical Analysis

A completely randomized design was used, and the data were evaluated using one-way analysis of variance (ANOVA) in SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The results were presented as the mean and standard error (SE) of the mean. Differences between treatment means were determined by Duncan's multiple comparison tests. Significance was assessed at $P < 0.05$.

3. Results

3.1. Sensory Evaluation of the Potato Vine Silage

The 15 different silage samples were evaluated based on three sensory parameters (color, odor, and texture). All the treatments, including the control, maintained a yellow-green color that was similar to the original color prior to silo storage. The samples had a mild sourness and slightly

alcoholic odor, and no unpleasant odor was detected. The silage was devoid of mildew and had maintained a good shape after 45 days of ensiling. The alcoholic taste of the FA and PFJ treatments was more prominent than the control, CF, and PP. The color of the potato vine ensilage without any additives was closer to yellow-brown.

3.2. Fermentation Quality Evaluation of the Potato Vine Silage

The effects of the different moisture contents and additives on the fermentation quality are indicated in Tables 3 and 5. Moisture and additives significantly influenced the silage pH, LA, AA, LA: AA, and $\text{NH}_3\text{-N}$ /total-N ($\text{NH}_3\text{-N/TN}$) ($P < 0.05$). In all silages, pH increased with decreasing moisture content, while, the concentration of LA was slightly reduced with decreasing moisture content. LA declined during the fermentation process, and only a relatively low amount of LA was detected in the FA, PFJ, CF, and PP treatments. No butyric acid (BA) was detected in the FA and PP treatments. LA content was highest and pH, AA, and BA were lowest at M1 compared to M2 and M3. In the control group, AA was higher than BA, and the concentrations of AA were lower in the treatment groups, with the exception of the CF treatments

(A3). AA increased with decreasing moisture content in A1, but decreased in A3 and A4. At M1, the lowest concentration of $\text{NH}_3\text{-N/TN}$ was observed with the addition of FA or PP. The $\text{NH}_3\text{-N/TN}$ content at M2 was lower with the addition of PFJ or CF than the M1 and M3 moisture contents.

At the three different moisture levels, the control treatments had the highest pH, BA, and $\text{NH}_3\text{-N/TN}$ values and the lowest LA contents. Furthermore, the pH, LA, and BA values differed significantly from the other four treatments ($P < 0.05$). The application of CF resulted in LA:AA ratios of 2.42, 2.15, and 1.75 at the three moisture levels, respectively, which was significantly lower than the other treatments. At M1, FA was associated with a significantly higher concentration of LA than the other treatments ($P < 0.05$). At M1, the pH values of A1 and A4 were 3.71 and 4.06, respectively (all of the values were lower than 4.20), which were significantly lower than A2 and A3 ($P < 0.05$). $\text{NH}_3\text{-N/TN}$ was only 0.17% DM at M1 with the addition of FA, which suggests that FA could reduce protein loss. At M2, $\text{NH}_3\text{-N/TN}$ in A1 was significantly lower than the other three treatments. At M3, $\text{NH}_3\text{-N/TN}$ in A2 and A3 was 1.95% DM and 2.41% DM, respectively, which was significantly higher than A1 and A4 ($P < 0.05$).

Table 3. Effects of moisture content and additives on the fermentation quality of potato vine silage.

Item	pH	LA (% DM)	AA	LA/AA	BA	$\text{NH}_3\text{-N/TN}$ (%)
M ₁ CK	4.92±0.05a	0.93±0.06e	0.65±0.03b	1.43±0.08b	0.16±0.07a	2.59±0.01a
M ₁ A ₁	3.71±0.04e	2.52±0.05b	0.09±0.07d	39.57±13.51a	-	0.17±0.01e
M ₁ A ₂	4.39±0.03c	1.47±0.09d	0.15±0.05c	6.48±0.45b	0.03±0.03b	2.35±0.04c
M ₁ A ₃	4.55±0.03b	3.56±0.07a	1.47±0.07a	2.42±0.04b	0.16±0.03b	2.52±0.02b
M ₁ A ₄	4.06±0.04d	2.12±0.06c	0.15±0.04d	10.29±1.43b	-	0.25±0.00d
M ₂ CK	5.13±0.24a	0.67±0.05e	0.67±0.04b	1.03±0.08c	0.53±0.04a	2.45±0.00a
M ₂ A ₁	4.14±0.14c	2.00±0.03b	0.41±0.00c	4.97±13.51b	-	0.93±0.00e
M ₂ A ₂	4.40±0.02b	1.46±0.07d	0.15±0.05d	3.00±0.45bc	0.03±0.04c	1.79±0.02c
M ₂ A ₃	4.60±0.02b	3.05±0.03a	1.42±0.05a	2.15±0.04c	0.16±0.04b	2.36±0.00b
M ₂ A ₄	4.45±0.01b	1.76±0.05c	0.15±0.06d	10.24±1.44a	0.07±0.06c	1.11±0.01d
M ₃ CK	5.20±0.02a	0.60±0.06e	0.68±0.08b	0.90±0.10b	0.57±0.04a	2.73±0.05a
M ₃ A ₁	4.25±0.03d	1.96±0.07b	0.41±0.09c	4.90±0.48b	-	1.02±0.00e
M ₃ A ₂	4.40±0.18cd	1.49±0.13d	0.59±0.08d	3.07±0.28b	0.03±0.02c	1.95±0.01c
M ₃ A ₃	4.67±0.04b	3.4±20.04a	1.34±0.06a	1.75±0.03b	0.16±0.05b	2.41±0.01b
M ₃ A ₄	4.44±0.02cd	1.78±0.05c	0.13±0.10d	13.96±3.75a	0.08±0.08bc	1.18±0.01d

Note: data are mean ± standard error; different small letters in the same column under the same moisture content indicate a significant difference ($P < 0.05$); - means not detectable.

3.3. Chemical Composition of the Potato Vine Silage

As shown in Table 4 and 6, significant interactions were found between moisture content and additives for DM, CP, NDF, ADF, and WSC after ensiling for 45 d ($P < 0.05$). For all of the treatments, DM, ADF, and NDF exhibited a slightly increased trend with decreasing moisture content, while CP remained relatively constant. Additionally, in the control

group, the WSC content in M2 (1.14% DM) was higher than in M1 and M3, and the CP content in M2 (17.54% DM) was lower than M1 and M3. In the A1 group, the WSC content in M1 was higher by 24.20% and 35.36% than M2 and M3, respectively. In the A3 and A4 treatments, the WSC content in M2 increased with decreasing moisture content.

Table 4. Effects of moisture content and additives on the nutritional components of potato vine silage.

Item	DM	CP (% DM)	NDF	ADF	WSC
M ₁ CK	27.80±0.17bc	17.71±0.11a	33.48±0.25a	29.22±0.35a	0.60±0.04e
M ₁ A ₁	26.24±1.28d	18.24±0.04a	27.39±0.08d	24.00±0.60d	4.67±0.12a
M ₁ A ₂	26.71±0.33cd	16.74±0.64b	29.70±0.54b	25.61±0.29c	1.06±0.08c

Item	DM	CP (% DM)	NDF	ADF	WSC
M ₁ A ₃	28.22±0.46b	16.19±0.35b	28.47±0.09c	27.08±0.04b	0.92±0.02d
M ₁ A ₄	33.48±0.19a	12.47±0.27c	29.26±0.22b	26.20±0.49b	2.51±0.01b
M ₂ CK	38.61±0.12b	17.54±0.62b	34.55±0.39a	30.99±0.64a	1.14±0.10e
M ₂ A ₁	37.16±0.16c	18.44±0.30a	29.76±0.36d	25.26±0.22d	3.76±0.02a
M ₂ A ₂	36.99±0.40c	17.63±0.17b	32.80±0.45b	26.78±0.14c	1.54±0.04c
M ₂ A ₃	38.49±0.03b	15.93±0.16c	32.41±0.29b	28.01±2.36b	1.44±0.00d
M ₂ A ₄	42.20±0.50a	13.44±0.11d	30.86±0.31c	26.37±0.23c	2.75±0.00b
M ₃ CK	47.41±0.14b	17.99±0.24ab	35.54±0.39a	31.14±0.38a	1.03±0.03e
M ₃ A ₁	46.34±0.28d	18.42±0.57a	30.61±0.28d	26.56±0.16d	3.45±0.03a
M ₃ A ₂	46.25±0.36d	18.34±0.07a	32.28±0.33c	27.47±0.35c	1.30±0.00d
M ₃ A ₃	46.91±0.34c	17.60±0.08b	33.50±0.40b	28.11±0.40b	1.47±0.04c
M ₃ A ₄	50.10±0.38a	14.22±0.12c	31.69±0.37c	26.75±0.34d	2.92±0.01b

At the three different moisture levels, the control treatments had the lowest WSC contents and the highest ADF and NDF contents, all of which differed significantly from those of the other four treatments ($P < 0.05$). The application of FA had the highest concentration of WSC compared with the other three treatments. The DM and CP contents with the addition of PP were significantly higher than the other four treatments, and ADF and NDF were significantly lower than the control group ($P < 0.05$). The WSC content in A1 was significantly higher than the control and other treatment groups ($P < 0.05$). At M1, the DM content in A1 was significantly lower than the control, A3, and A4 ($P < 0.05$). The CP content in A4 was significantly lower than the other treatments ($P < 0.05$). The WSC content in A4 was significantly higher than the control at 318.33% ($P < 0.05$). At M2, the concentration of WSC in A1 (3.76%) and A4 (2.75%) was significantly higher compared to the control,

A2, and A3. In addition, the concentration of CP in A4 was lower than the control, A2, and A3. At M3, the WSC content in A4 was higher than the control by 183.50%, and CP was lower than the control by 20.96%.

3.4. Glycoalkaloids in the Potato Vine Silage

The effects of different moisture contents and additives on glycoalkaloids in the potato vine silage are presented in Table 5. The glycoalkaloid content of the potato vine silage increased with decreasing moisture content. At the three moisture levels, the glycoalkaloid concentrations in the control group were 19.83 mg 100 g⁻¹, 32.34 mg 100 g⁻¹, and 40.94 mg 100 g⁻¹, respectively, which were significantly higher than the treatment groups ($P < 0.05$). The solanine content in M1 was the lowest among all the treatments.

Table 5. Effects of moisture content and additives on glycoalkaloid content in potato vine silage (mg 100 g⁻¹ DM).

Item	Control	A1	A2	A3	A4
M1	19.83a±0.35	0.55e±0.40	4.23c±0.15	12.60b±0.23	3.32b±0.38
M2	32.34a±0.23	4.57d±0.26	6.09c±0.37	15.28b±0.47	6.46c±0.48
M3	40.94a±0.11	7.73d±0.17	11.06c±0.19	19.22b±0.24	10.82c±0.27

The FA, PFJ, CF, and PP treatments exhibited significantly decreased solanine concentrations ($P < 0.05$), and the addition of FA was associated with the lowest solanine content of only 0.55 mg 100 g⁻¹ (in M1). At M2 and M3, the

glycoalkaloid contents in A2 and A4 were significantly lower than the control ($P < 0.05$); however, glycoalkaloid content did not differ significantly between A2 and A4.

Table 6. ANOVA of moisture content, additives, and their interaction.

Item	pH	LA	AA	LA/AA	BA	NH ₃ -N/TN	DM	CP	NDF	ADF	WSC	PG
MC	44.06*	185.92*	14.57*	278.50*	0.93	587.44*	0.04*	50.58*	63.98*	332.93*	22.14*	484.70*
A	102.99*	802.13*	706.48*	397.12*	28.72*	0.00*	234.42*	467.76*	86.05*	92.06*	0.06*	1441.93*
MC×A	13.83*	49.25*	10.11*	236.77*	0.93**	855.01*	7.21*	7.73*	6.17*	13.13*	245.50*	42.07*

Note: * indicates significant difference at $P < 0.05$.

MC, moisture content; A, additives.

4. Discussion

4.1. Chemical Composition of the Pre-ensiled Materials

Silage quality is influenced by the raw materials, moisture content, and the compaction degree [33, 34]. In addition, lower buffering capacity, and a certain amount of substrate (WSCs) should also be provided for lactic acid bacterial fermentation [35, 36]. Meeske et al. [37] reported that the pH

value of ensiled corn with 107 g·kg⁻¹ DM WSC could be reduced to less than 4.0 in two days. In the present study, pre-ensiled potato vines had a low WSC (3.68% DM, which is less than the minimum recommended level for ensiling) [38, 39], higher buffering capacity (694.11 mE kg⁻¹ DM), and high moisture content (85%–92%) [40], all of which are considered to render ensiling more difficult. McDonald et al. [38] showed that a low WSC accompanied by a high buffering capacity may result in a high pH in legume silage.

A high pH value during ensiling does not create the optimal environment for the growth of aerobic microorganisms. Dunière *et al.* [41] reported that an adequate DM (30–40 g kg⁻¹), lactic acid bacteria (LAB), and an initial WSC of more than 70 g kg⁻¹ were required for satisfactory fermentation. An effective way to obtain satisfactory fermentation is to supplement with different additives that are highly soluble or can reduce pH rapidly, such as corn flour or organic acids.

4.2. Effects of Moisture Content on the Fermentation Quality of Potato Vine Silage

Moisture is the main factor influencing the fermentation quality of ensiled forage. Moisture is required by LAB for metabolic reactions and has a significant effect on the initial level and the transportation of oxygen during the ensilage process [42]. As critical pH values vary with moisture content [38], fermentation under higher moisture contents is accompanied by a lower critical pH, and the growth of aerobic microorganisms is difficult to restrain under a low pH, even at pH values below 4.0 [43]. When the moisture content is low, the remaining residual air will increase the risk of the proliferation of undesirable microorganisms [44].

In this study, a declining tendency in fermentation quality was observed with decreasing moisture content, as indicated by the high pH and low LA content of the ensilage. This corroborates the studies of Kim *et al.* [45] and Manyawu *et al.* [46]. A moisture content of 75% produced the best quality silage in this study. The silage treated with FA at 75% water content was well-preserved as indicated by the good fermentation characteristics, such as a lower pH value, lower NH₃-N/TN, less WSC loss, and the absence of BA (Table 3). However, the concentration of CP at 55% moisture content was higher than at 75%. The high variation in CP content observed was mostly likely a result of endogenous enzyme activity at the different moisture levels. Lower moisture conditions in raw materials that have been dried or wilted inhibit microbial activity, influence proteolysis in silages by restricting protein degradation [47] and enhance CP in CF-treated and PFJ-treated silages. The results from the present study suggested that the method of partial drying followed by ensiling may be an approach for biomass storage and stabilization.

4.3. Effects of Additives on the Fermentation Quality of Potato Vine Silage

At the three different moisture contents, all the treatments (FA, PFJ, CF, and PP) could improve the potato vine silage quality by enhancing the sensory quality and reducing the pH of the ensiled forage. Significant differences were observed in the BA generated at all moisture content levels ($P < 0.05$). High concentrations of both BA and NH₃-N/TN were found in the CF-treated silages. The absence or relatively low amount of BA in the other treatments (FA, PFJ, and PP) indicated that the activity of *Clostridium butyricum*, which is produced during fermentation was reduced. Kung and Stokes [48] suggested that a LA to AA ratio of more than 3:1 would

be an indication of more dominant homolactic fermentation. The LA: AA ratios with the addition of CF were 2.42, 2.15, and 1.75, respectively, which indicates that heterofermentative LAB dominated the ensiling system at the three different moisture contents, and the heterofermentative LAB process was advanced by the addition of CF. Alli *et al.* [49] found that molasses could facilitate heterofermentative LAB and result in the partial fermentation of LA to AA. Kung *et al.* [50] showed that treatment with ammonia could decrease the LA: AA ratio and increase the NH₃-N concentration during ensiling. This may explain why high concentrations of both BA and NH₃-N/TN and a low WSC content were found in the CF-treated silages. The amount of ADF and NDF is an important variable influencing the fiber quality of the feed. The amount of ADF is a major factor affecting silage energy composition. Silage with a lower ADF concentration is more digestible and has greater nutritional value [51]. The concentration of ADF and NDF was significantly decreased with the addition of PP ($P < 0.05$). The partial degradation of cell walls during ensiling may account for the lower NDF and ADF contents in the potato pulp.

The addition of PFJ to silage suppresses mold growth [52]. Cao *et al.* [53] showed that the addition of PFJ to baled alfalfa silage could reduce the pH value from 5.66 to 4.66 compared with no additive. In this study, the addition of PFJ resulted in the pH of the ensiled forage stabilizing to approximately 4.40 after 45 d, which was indicative of increased dominant homolactic fermentation. This finding was in agreement with the results of Song *et al.* [24].

In the current study, the CP contents of the fresh PV was 18.49% DM, which was comparable with the CP in alfalfa, corroborating the studies of Salehi *et al.* [23], Luo and Guo [40], and Muck *et al.* [3]. Potato vines constitute a potential protein source in ruminant diets and can partially replace protein fodder [22, 54]. The CP contents of the control treatment were lower by approximately 3.5% compared with the FA-treated PV. The reduction in CP content could be attributed to nitrogen losses from protein degradation, which would explain why the addition of FA facilitates the ensiling process. FA resulted in good-quality silage with lower pH and NH₃-N/TN values and a higher WSC content, and was previously found to rapidly reduce pH and restrict the reproduction of putrefying bacteria, such as *Clostridium* [55], as well as depress the activity of proteolytic enzymes [16]. Furthermore, the inclusion of FA preserved the soluble carbohydrates from the original material or those produced by polysaccharide conversion, and lead to the formation of microorganism protein [16], which is beneficial for ruminants.

4.4. Effects of Potato Glycoalkaloids on Potato Vine Silage

Potato vines are potential fodder sources; however, they are generally discarded as waste due to the presence of toxic glycoalkaloids, which are harmful to ruminants when provided in large quantities [7, 56–57]. König [20] reported that the toxic glycoalkaloid dose was typically 225 mg

100g⁻¹ body weight for sheep. The adverse effect of potato glycoalkaloids in animals is related to their anti-cholinesterase activity in the central nervous system, cellular membrane disruption, induction of liver enzymes, teratogenicity, and embryotoxicity [58, 59].

In the present study, both wilting and ensiling reduced the concentration of the anti-nutritional components of potato vine. The potato glycoalkaloid content of the fresh potato vine was 107.06 mg 100 g⁻¹ DM and was reduced to 19.83, 32.24, and 40.94 mg 100g⁻¹ DM, respectively, at 75%, 65%, and 55% moisture contents after 45 days of ensiling. The toxicity levels of the treated silages (FA, PFJ, CF, and PP) ranged from 0.55 to 19.22 mg 100 g⁻¹ DM, with glycoalkaloid degradation rates of 53.04% to 97.23%. This indicated that the majority of the glycoalkaloids were degraded during the potato ensiling process, which suggested that the silage might be safe for consumption by ruminants. This finding is consistent with that reported by Malecky *et al.* [22]. In the present study, the addition of FA at 75% moisture content resulted in the fastest degradation rate, while the slowest degradation was found in the CF-treated silage at 55% moisture content. The degradation levels appear to be correlated with the fermentation quality. Due to the fungal toxic properties of potato glycoalkaloids [60], it is necessary that LAB growth is improved in order to create an acidic environment. It can be concluded that glycoalkaloid degradation is associated with the transformation process of microbial degradation during acid-catalyzed hydrolysis [61]. Ensiling thus constitutes an effective means of reducing the glycoalkaloid content in potato vine.

5. Conclusion

Moisture content significantly influenced silage quality, and partial wilting followed by ensilage may improve the fermentation characteristics of fresh forage. Untreated potato vines do not stored well, and ensiling with additives (formic acid, pre-fermented juices and potato pulp) improved the fermentation quality of the silage with lower pH value and NH₃-N/TN contents, and higher CP and WSC contents. The glycoalkaloid concentration of the potato vines was most effectively reduced by ensiling with formic acid, followed by the addition of pre-fermented juices and potato pulp. In conclusion, a moisture content of 75% + formic acid treatment performed the best. Based on the findings of this study, ensiling with additives is an effective and safe storage technique for preserving potato vines. Furthermore, protein fodder may be partially substituted by PV silage treated with additives without any adverse effect for ruminants, and can thus be utilized as a source of livestock feedstuff.

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