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FERMENTATION AND MICROBIOLOGICAL CHARACTERISTICS OF MILLET SILAGE ACCORDING TO VARIETY, HARVEST STAGE AND SALT ADDITION

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ABSTRACT

In Niger, a Sahelian country facing significant climate variability, preserving millet residues through ensiling represents a promising solution to address seasonal forage shortages. The objective of this study was to assess the influence of variety (six genotypes), harvest stage (flowering and maturity), and 1 % salt addition on the fermentation and microbiological quality of these silages. The results show that these three factors significantly influence key parameters. The harvest stage has the most pronounced effect. Flowering silages exhibit a lower pH and higher levels of acetic, propionic, and butyric acids, indicating more active fermentation but also an increased risk of clostridial growth. Variety particularly affects organic acid levels, with the Maywa variety generally producing more acetic, propionic, and butyric acids. Salt addition has a moderate but significant effect, reducing the overall pH and increasing acetic and propionic acid levels. Microbiological analyses revealed the consistent presence of lactic acid bacteria, butyric acid bacteria, and spoilage flora. The addition of salt tended to reduce the populations of butyric acid bacteria and spoilage flora, although not significantly. Significant correlations confirmed the expected links, notably the acidifying effect of lactic acid and the positive association between propionic and butyric acids. In conclusion, for quality silage in Niger, it is recommended to favor a harvest stage close to maturity to minimize undesirable fermentations, to select varieties such as Siaka Millet associated with a low pH, and to consider adding 1% salt as a simple additive to improve acidification and positively modulate the fermentation profile.

Keywords: Anaerobic fermentation, Animal feed, Fermentation profile, Microorganisms, Silage quality, Niger

INTRODUCTION

In Niger, a Sahelian country characterized by a long dry season and high rainfall variability, the availability of quality forage is a major constraint on livestock productivity, a key pillar of the economy and food security **(Hiernaux *et al.*, 2015; CORAF, 2022)**. In this context, forage conservation through ensiling, although underutilized, appears to be a promising strategy for mitigating seasonal livestock feed deficits **(Moussa *et al.*, 2017; Korombé *et al.*, 2023a; Korombé, 2024)**. Crop residues, particularly those of millet (*Pennisetum glaucum*), are also a significant factor in this regard; the country's staple cereal represents an abundant and undervalued resource. Its transformation into silage could significantly contribute to the resilience of agro-pastoral systems **(Chaïbou *et al.*, 2012; Amole and Ayantundé, 2016; Korombé, 2024)**.
However, the technical success of silage made from these residues in Niger depends on controlling critical fermentation parameters, in an environment where local knowledge is limited and commercial additives are often difficult to access. The quality criteria for good silage are universal: a rapid drop in pH due to dominant lactic acid production, and minimal levels of ammonia and butyric/propionic acids, indicators of undesirable anaerobic fermentation **(McDonald *et al.*, 1991; Pahlow *et al.*, 2003)**. On the other hand, the initial composition of the forage, which determines the course of fermentation, is itself influenced by other factors **(Amyot, 2003)**.
The choice of millet variety is a key initial factor. Improved varieties and local ecotypes likely differ in their soluble sugar content, dry matter, buffering capacity and key parameters for fermentation, as has been demonstrated for other crops **(Contreras- Govea *et al.*, 2011)**. The harvest stage of the residues drastically affects their moisture content and composition, with an increased risk of poor (butyric) fermentation when dry matter is too low **(Kung and Shaver, 2001)**. Finally, the use of simple, non-toxic, affordable, and locally available additives, such as table salt (NaCl), could offer a pragmatic solution for improving preservation **(McLaughlin *et al.*, 2002; Johansson, 2008; Korombé *et al.*, 2023a)** by inhibiting harmful bacteria and modulating the microbial flora, as suggested by similar studies **(Ergin and Gümüş, 2020)**.
The interaction between these three factors (variety, harvest stage, and salt addition) regarding the fermentation and microbiological quality of millet residue silage remains largely unexplored within the context of Niger. Consequently, this study seeks to address this research gap. Its objective is to systematically assess the effect of these factors on chemical (pH, ammonia, organic acids) and microbiological (lactic acid bacteria, butyric acid bacteria, spoilage flora) parameters in order to propose suitable and accessible technical recommendations for Nigerien farmers to improve the utilization of millet residue through silage.

MATERIAL AND METHODS

Study area

The study was conducted in Niger, at the Sadoré experimental station of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT Sahelian Centre), located in the rural commune of Youri (Kourtéré), Kollo Department, Tillabéri Region. Located at coordinates 13°14'N and 2°16'E, the Sadoré experimental station features a climate defined by a late rainy season, typically spanning from June to October. This is followed by a dry season from November to May, which includes a notably intense hot period between March and May. The station receives an average annual rainfall of 562 mm, and temperatures with temperatures fluctuating between 12°C and 44°C and a mean temperature of 29.4°C **(Umutoni *et al.*, 2021)**. During the trial period, the mean e minimum and maximum temperatures were 22.85±4.05°C and 36.42±3.91°C, respectively, resulting in an overall average of 29.64±3.51 °C. Total annual precipitation reached 715.29 mm.

Experiment design

The experiment followed a completely randomized design, focusing on the evaluation of three factors:

- the millet variety, with 6 modalities consisting of 4 improved dual-use varieties Chakti, ICMV167005 (Siaka Millet), ICMV167006 (ICRI-Tabi), ICMH177111 (Alambana) and two local varieties (Maywa and Locale Sadoré);
- the cutting stage: with the flowering stage, where the plant is harvested whole to then be ensiled and the maturity stage, where the grain is harvested first for human consumption, followed by the harvesting of the residual biomass;
- the addition of salt, compared across two levels: a control group (no salt) and salt addition at a rate of 1% of the fresh ensiled biomass mass **(Tamboura *et al.*, 2005)**.

The combination of the different modalities tested (6x2x2) yielded 24 treatments. Each treatment was repeated 3 times, resulting in 72 repetitions for the entire study.

Silage- making process

The silage was produced from millet crops grown between June 29 to November 14, 2019, at the Sadoré station.

The harvested biomass was first chopped into small pieces (2 to 5 cm) using a Seth Tufail Toka Machine (model 37397) shredder, following the methods described by **Morales *et al.*, 2015** and **Trevisoli *et al.*, 2017**. Subsequently, for each variety and growth stage (flowering and maturity), an initial weight (Pi) of 20 kg of biomass was packed into plastic bags **(Morales *et al.*, 2011)**. Following this treatment, sodium chloride (NaCl) was incorporated. The biomass was then manually compacted in successive layers to ensure density. To maintain optimal anaerobic conditions, each well-compacted bag was inserted into a second protective bag. These units were hermetically sealed using twine and adhesive tape, then stored under anaerobic conditions at room temperature for a 60 days period **(Costa *et al.*, 2012; Lyimo, 2017)** within a well-ventilated, enclosed facility. Fermentation progress was monitored through daily inspections of the bags and assessment of the emitted odors.

Sampling

Representative samples were collected both prior to bagging and then upon opening the silage after the 60 days of storage period. These samples were oven-dried at 65°C for 72 hours to determine the dry matter content, according to the protocol described by **Trevisoli *et al.*, 2017**. Additionally, sub-samples were collected at the time of opening of the silage and immediately stored at -20 °C for subsequent analysis of fermentation quality and microbiological parameters.

Determination of fermentation parameters

pH measurement and determination of ammonia and organic acid content

The determination of pH, ammonia nitrogen (NH3-N), and organic acid concentrations was conducting at the National Laboratory of Public Health and Expertise of Niger (LANSPEX).

To measure the pH and ammonia levels, a 10 g aliquot of fresh silage was diluted in 100 ml of distilled water. The resulting suspension was then homogenized for 5 minutes using a blender. After filtration, the pH was measured using a WTW Multi 9620 IDS pH meter (Hag *et al.*, 1982). Ammonia was measured using a Hach Lange DR 3900 spectrophotometer, according to the Nessler method **(Hach Company, 2017)**. However, the initial extract (10 g of silage in 100 ml of distilled water) could not be analyzed directly, as the ammonia concentrations exceeded the instrument's detection range of 0.02 to 2.50 mg/L NH3-N. Consequently, a second dilution was performed by adding 1 ml of the initial extract to 25 ml of distilled water.

Organic acids (lactic acid, acetic acid, propionic acid, and butyric acid) were quantified via HPLC using an Agilent Infinity 1260 equipped with an isocratic pump. While the procedure followed the general protocol described by **Kim *et al.* (2017)**, it was modified for this study: the mobile phase consisted of 0.1% phosphoric acid in distilled water under isocratic conditions rather thangradient elution.

Determination of microbiological parameters

Microbiological analyses were conducted at the Central Livestock Laboratory of Niger (LABOCEL).

The enumeration and identification of various microorganisms across all samples followed the ISO 7218-2007 standard, which establishes general requirements and guidance for microbiological examinations throughout the entire analytical process **(ISO, 2007)**. It should be noted that, for the detection and enumeration of yeasts and molds, the medium used is Chloramphenicol Sabouraud. Clostridium perfringens and sulfite-reducing anaerobic bacteria were counted on TSC (Tryptose Sulfite Cycloserine) agar, and lactic acid bacteria were counted on MRS (Man Rogasa Sharp) agar.

Statistical analysis of data

Quantitative data regarding to fermentation and microbiological parameters were subjected to a three-way Analysis of Variance (ANOVA) using a 6x2x2 factorial design (Variety, Cutting Stage, and Salt Addition). All analyses were performed using the GLM procedures in SPSS 20.0. For factors with two levels (salt addition and cutting stage), pairwise comparisons of means were conducted at a 5% significance level ($p<0.05$) using the Least Significant Difference (LSD) test. For the variety factor, which included more than two levels, Tukey's HSD test was employed for post-hoc comparisons. It should be noted that, to evaluate the effect of the different factors on the measured parameters, the variety, cutting stage, and salt addition were considered fixed factors, and the fermentation and microbiological parameters studied were considered dependent variables. Thus, the specific effects of the factors, as well as those of their interactions, were determined.

Correlation tests were carried out to determine the different types of relationships between the variables.

RESULTS AND DISCUSSION

Results

Effect of variety and harvest stage on organic acid profiles in millet silage

Silages harvested at the flowering-stage exhibited significant variations in pH, ammonia (NH3), Lactic Acid (LA), and Propionic Acid (PA) concentrations across the different varieties (Table 1). Regarding pH, only the Chakti variety showed significantly higher values ($p<0.05$) compared to the other varieties. Furthermore, NH3 levels in Maywa silages were significantly higher than those recorded for Alambana, whereas no significant differences were observed among the remaining varieties.

Regarding LA, the levels measured with ICRI-Tabi and Locale Sadoré were statistically higher than those obtained with Alambana and Chakti. Alambana and Siaka millet silages produced PA levels that were statistically lower than those of Maywa silages.

At maturity, significant differences were found depending on the variety for all parameters studied except for LA (Table 1). Chakti, Siaka Millet, and Locale Sadoré silages were characterized by statistically lower pH values compared to ICRI-Tabi and Alambana, while Maywa silages had significantly lower pH values than ICRI-Tabi but higher than Chakti. For NH3, the Locale Sadoré and Maywa varieties showed significantly higher values than Chakti, ICRI-Tabi, and Siaka Millet. Regarding AA (Acetic Acid), only the level obtained in Maywa was statistically higher than that found in Alambana. PA values statistically higher than those of Siaka Millet, Locale Sadoré, and ICRI-Tabi silages were recorded in Maywa silages. The latter produced silage with significantly higher Butyric Acid (BA) content than Locale Sadoré.

Analysis of the variety means (average of the two harvest stages for each parameter) shows significant differences between varieties for all parameters except LA (Table 1). The Siaka Millet variety exhibited the lowest pH and ammonia levels. The Maywa variety, on the other hand, recorded the highest levels of AA, PA, and BA.

Analysis of the effects of the harvest stage shows significant variations for all parameters studied except for LA (Table 1). Silages at the flowering stage are characterized by lower pH and ammonia levels and higher levels of AA, PA and BA compared to silages at the maturity stage.

As for the interactions between varieties and cutting stages, they are statistically significant for all parameters (Table 1).

Microbiological parameters of millet straw silage based on variety and harvest stage

The presence of Lactic acid Bacteria (LAB), Butyric Acid Bacteria (BAB), and Spoilage Flora (SF) was noted in the silage of all varieties at both cutting stages (Table 2).

For silage harvested at the flowering stage, only the number of LAB varied significantly depending on the variety. The averages recorded for Alambana and Siaka Millet were statistically lower than those of the other varieties.

Regarding silage at the maturity stage, the average SF obtained at Chakti and Locale Sadoré are significantly lower than those of Maywa and ICRI-Tabi.

The analysis, of the averages of the two cutting stages according to the variety, showed a significant variation only for the number of LAB. For this parameter, the silages of the ICRI-Tabi variety recorded the highest average while the lowest average was obtained in the Alambana silages.

Analysis of parameter variations according to harvest stage shows no significant differences. However, a significant interaction between variety and cutting stage is observed for the number of LAB (Table 2).

Table 1 Effects of variety and phenological stage at harvest on the organic acid profile of silage

Harvest stage	Parameters						
	Varieties	pH	NH3 (%)	LA (%)	AA (%)	PA (%)	BA (%)
Flowering	Alambana	3.96^{b}	0.03^{b}	6.68^{b}	1.48^{a}	1.03^{b}	1.71^{a}
	Shakti	4.48^{a}	0.05^{ab}	6.31^{b}	1.76^{a}	1.41^{ab}	1.99^{a}
	ICRI-Tabi	3.82^{b}	0.05^{ab}	11.24^{a}	1.78^{a}	1.58^{ab}	2.20^{a}
	Local Sadoré	4.05^{b}	0.04^{ab}	9.95^{a}	1.53^{a}	1.28^{ab}	1.72^{a}
	Maywa	4.07^{b}	0.05^{a}	8.35^{ab}	2.07^{a}	1.86^{a}	2.29^{a}
	Mil de Siaka	3.89^{b}	0.04^{ab}	9.22^{ab}	1.65^{a}	0.98^{b}	1.64^{a}
	SEM	0.057	0.003	1.053	0.133	0.131	0.171
	p-Value	0.000	0.015	0.043	0.083	0.004	0.079
	Avg HS	4.05	0.04	8.63	1.71	1.36	1.93
Maturity	Alambana	4.54^{ab}	0.05^{ab}	7.75^{a}	0.52^{b}	1.20^{ab}	0.90^{ab}
	Shakti	4.12^{d}	0.04^{b}	7.18^{a}	1.18^{ab}	1.05^{abc}	1.37^{ab}
	ICRI-Tabi	4.81^{a}	0.04^{b}	7.10^{a}	0.79^{ab}	0.66^{bc}	0.56^{ab}
	Local Sadoré	4.24^{cd}	0.06^{a}	12.09^{a}	1.39^{ab}	0.45^{c}	0.46^{b}
	Maywa	4.45^{bc}	0.06^{a}	7.68^{a}	1.74^{a}	1.42^{a}	1.77^{a}
	Mil de Siaka	4.17^{cd}	0.04^{b}	5.40^{a}	1.13^{ab}	0.49^{c}	0.52^{ab}
	SEM	0.062	0.006	1.742	0.234	0.224	0.264
	p-Value	0.000	0.027	0.220	0.038	0.045	0.021
	Avg HS	4.39	0.05	7.87	1.13	0.88	0.93
Avg Var	Alambana	4.25^{a}	0.04^{ab}	7.22^{a}	1.00^{b}	1.12^{ab}	1.31^{b}
	Shakti	4.30^{a}	0.04^{ab}	6.75^{a}	1.47^{ab}	1.23^{ab}	1.68^{ab}
	ICRI-Tabi	4.31^{a}	0.04^{ab}	9.17^{a}	1.29^{b}	1.12^{ab}	1.38^{ab}
	Local Sadoré	4.15^{ab}	0.05^{ab}	11.02^{a}	1.46^{ab}	0.87^{b}	1.09^{b}
	Maywa	4.26^{a}	0.05^{a}	8.01^{a}	1.91^{a}	1.64^{a}	2.03^{a}
	Mil de Siaka	4.03^{b}	0.04^{b}	7.31^{a}	1.39^{ab}	0.74^{b}	1.08^{b}
	SEM	0.042	0.003	1.018	0.134	0.13	0.157
	p-Value	0.000	0.014	0.061	0.003	0.001	0.002
Levels of significance	HS	***	*	NS	***	***	***
	Var x HS	***	**	*	**	**	**

NB: Depending on the harvest stage, in the lines, the averages bearing at least one identical letter as a superscript are not statistically different from each other.
Legend: NH3 – Ammonia, **LA** – Lactic Acid, **AA** – Acetic Acid, **PA** – Propionic Acid, **BA** – Butyric Acid, **Var** – Variety, **HS** – Harvest Stage, **Var x HS** –Interaction between variety and Harvest stage, **Avg Var** – mean of the parameter for the two cutting stages per variety, **Avg HS** – Means of the parameter for all varieties per harvest stage, **SEM** – Standard Error of the Mean, **NS** – Not Significant; *** – Significant at the 0.001 level, ** – Significant at the 0.01 level, * –Significant at the 0.05 level.

Table 2 Effects of variety and harvest stage on the microbiological parameters of silage

Harvest stage	Parameters			
	Varieties	BAB (log CFU/g)	LAB (log CFU/g)	SF (log CFU/g)
Flowering	Alambana	2.03 ^{a}	ND	4.44^{a}
	Shakti	3.81 ^{a}	3.91^{a}	4.59^{a}
	ICRI-Tabi	3.80 ^{a}	4.30^{a}	2.69^{a}
	Local Sadoré	3.23^{a}	3.70^{a}	4.72 ^{a}
	Maywa	2.26^{a}	4.08^{a}	3.03 ^{a}
	Mil de Siaka	2.31^{a}	1.18^{b}	3.96 ^{a}
	SEM	0.819	0.511	0.857
	p-Value	0.471	0.000	0.460
	Avg HS	2.91	2.86	3.91
Maturity	Alambana	2.88 ^{a}	4.10 ^{a}	4.90^{abc}
	Shakti	1.97 ^{a}	2.25 ^{a}	4.11^{c}
	ICRI-Tabi	2.42 ^{a}	4.37 ^{a}	5.08^{ab}
	Local Sadoré	1.87 ^{a}	3.93^{a}	4.13^{c}
	Maywa	2.57 ^{a}	2.36^{a}	5.15^{a}
	Mil de Siaka	3.89 ^{a}	3.78^{a}	4.26^{bc}
	SEM	0.81	0.73	0.273
	p-Value	0.555	0.235	0.044
	Avg HS	2.6	3.46	4.61
Avg Var	Alambana	2.45^{a}	2.05^{b}	4.67^{a}
	Shakti	2.89^{a}	3.08^{ab}	4.35^{a}
	ICRI-Tabi	3.11^{a}	4.33^{a}	3.89^{a}
	Local Sadoré	2.55^{a}	3.81^{ab}	4.42^{a}
	Maywa	2.42^{a}	3.22^{ab}	4.09^{a}
	Mil de Siaka	3.10^{a}	2.48^{ab}	4.11^{a}
	SEM	0.576	0.446	0.45
	p-Value	0.905	0.016	0.851
Levels of significance	HS	NS	NS	NS
	Var x HS	NS	***	NS

NB: Depending on the harvest stage, in the lines, the averages bearing at least one identical letter as a superscript are not statistically different from each other.
Legend: BAB – Butyric Acid Bacteria, **LAB** – Lactic Acid Bacteria, **SF** – Spoilage Flora, **Var** – Variety, **HS** – Harvest Stage, **Var x HS** – Interaction between variety and harvest stage, **Avg Var** – mean of the parameter for the two harvest stages per variety, **ND** – **Not Detected, Avg HS** – Means of the parameter for all varieties per harvest stage, **NS** – Not Significant, **SEM** – Standard Error of the Mean, **CFU** – Colony Forming Units, *** – Significant at the 0.001 level, ** – Significant at the 0.01 level, *– Significant at the 0.05 level.

Effects of salt and harvest stage on silage organic acid content

In flowering stage silage, only the pH showed a statistically significant variation (p-value < 0.05) depending on the addition of salt (Table 3). The pH of the silage with salt was lower compared to that of the control silage.

Regarding the silage at the maturity stage, no significant difference was recorded depending on the addition of salt for any of the parameters studied (Table 3).
Overall, the analysis of the average salt addition values shows a significant variation in pH, Acetic Acid (AA), and Propionic Acid (PA) values (Table 3). For example, silages treated with salt had lower pH values than control silages, while the opposite was true for AA and PA levels.

The interaction between variety and salt addition is statistically significant for pH, Lactic Acid (LA), PA, and Butyric Acid (BA) (Table 3). The interaction between salt addition and cutting stage is significant only for pH. Significant interactions between salt addition, variety, and cutting stage are observed for pH and LA (Table 3).

Table 3 Influence of salt addition and variety on the fermentation en-products of silage across multiple harvest stages

Harvest stage	Parameters	Adding salt	Average	SEM	p-Value
Flowering	pH	With salt	3.90	0.05	0.044
		Without Salt	4.05		
	Ammonia (%)	With salt	0.04	0.00	0.706
		Without Salt	0.04		
	LA (%)	With salt	9.24	0.52	0.413
		Without Salt	8.63		
	AA (%)	With salt	1.75	0.07	0.709
		Without Salt	1.71		
	PA (%)	With salt	1.72	0.13	0.052
		Without Salt	1.36		
	BA (%)	With salt	2.12	0.10	0.181
		Without Salt	1.93		
Maturity	pH	With salt	4.39	0.07	0.961
		Without Salt	4.39		
	Ammonia (%)	With salt	0.05	0.00	0.799
		Without Salt	0.05		
	LA (%)	With salt	7.90	0.74	0.978
		Without Salt	7.87		
	AA (%)	With salt	1.33	0.12	0.245
		Without Salt	1.13		
	P A (%)	With salt	1.04	0.13	0.388
		Without Salt	0.88		
	BA (%)	With salt	1.05	0.16	0.590
		Without Salt	0.93		
Avg SA	pH	With salt	4.15	0.017	0.007
		Without Salt	4.22		
	Ammonia (%)	With salt	0.04	0.001	0.989
		Without Salt	0.04		
	LA (%)	With salt	8.57	0.334	0.499
		Without Salt	8.25		
	AA (%)	With salt	1.54	0.047	0.072
		Without Salt	1.42		
	PA (%)	With salt	1.38	0.062	0.005
		Without Salt	1.12		
	BA (%)	With salt	1.59	0.056	0.050
		Without Salt	1.43		
			SA x Var	HS x SA	SA x Var x HS
Interactions	pH	With salt	***	**	***
		Without Salt			
	Ammonia (%)	With salt	NS	NS	NS
		Without Salt			
	LA (%)	With salt	*	NS	**
		Without Salt			
	AA (%)	With salt	NS	NS	NS
		Without Salt			
	PA (%)	With salt	*	NS	NS
		Without Salt			
	BA (%)	With salt	**	NS	NS
		Without Salt			

Legend: NH3 – Ammonia, **LA** – Lactic Acid, **AA** – Acetic Acid, **PA** – Propionic Acid, **BA** – Butyric Acid, **Var** – Variety, **HC** – Harvest Stage, **SA x Var** – Interaction between salt addition and variety; **HS x SA:** Interaction between harvest stage and Salt Addition; AS x Var x HS: Interaction between salt addition, variety, and harvest stage, **Avg SA** – Mean parameter for both harvest stages with salt addition, **SEM** – Standard Error of the Mean, **NS** – Not Significant, *** – Significant at the 0.001 level, ** – Significant at the 0.01 level, * – Significant at the 0.05 level.

Effects of salt addition on microbial profiles in silage at different harvest stages

In all silage samples (flowering and maturity), salt addition did not significantly influence microbiological parameters (Table 4). However, a general trend of dominance of LAB (Lactic Acid Bacteria) was observed in silage with salt (flowering) and of BAB (Butyric Acid Bacteria) and SF (Spoilage Flora) in silage without salt (flowering and maturity).

The overall analysis (Avg SA) of the effects of salt addition revealed a broad presence of all microbial groups within the silage, though no significant differences were observed (Table 4). Furthermore, the interaction between variety and salt addition, as well as all other tested interactions, failed to significantly influence the fermentation parameters (Table 4).

Table 4 Effects of salt addition on microbiological parameters according to the cutting stage

Harvest stage	Parameters	Adding salt	Average	SEM	p-Value
Flowering	BAB (log CFU/g)	With salt	2.38	0.33	0.270
		Without Salt	2.91		
	LAB (log CFU/g)	With salt	3.21	0.40	0.541
		Without Salt	2.86		
	SF (log CFU/g)	With salt	3.39	0.37	0.331
		Without Salt	3.91		
Maturity	BAB (log CFU/g)	With salt	1.84	0.35	0.137
		Without Salt	2.60		
	LAB (log CFU/g)	With salt	2.97	0.39	0.378
		Without Salt	3.46		
	SF (log CFU/g)	With salt	4.23	0.29	0.361
		Without Salt	4.61		
Avg SA	BAB log CFU/g	With salt	2.11	0.24	0.069
		Without Salt	2.75		
	LAB (log CFU/g)	With salt	3.09	0.20	0.800
		Without Salt	3.16		
	SF (log CFU/g)	With salt	3.81	0.25	0.208
		Without Salt	4.26		
			SA x Var	SA x HS	SA x Var x HS
Interactions	BAB (log CFU/g)	With salt Without Salt	NS	NS	NS
	LAB (log CFU/g)	With salt Without Salt	NS	NS	NS
	SF (log CFU/g)	With salt Without Salt	NS	NS	NS

Legend: BAB – Butyric Acid Bacteria, **LAB** – Lactic Acid Bacteria, **SF** – Spoilage Flora, **Var** – Variety, **HS** – Harvest Stage, **SA x Var** – Interaction between salt addition and variety, **SA x HS** – Interaction between salt addition and harvest stage, **SA x Var x HS** – Interaction between salt addition, variety and harvest stage; **Avg SA** – Average salt addition, **SEM** – Standard Error of the Mean, **NS** – Not Significant, **CFU** – Colony Forming Units, *** – Significant at the 0.001 level, ** – Significant at the 0.01 level, * – Significant at the 0.05 level.

Relationships between organic acid content and microbiological parameters of silage

Correlation Analysis between fermentation and microbiological parameters revealed several significant relationships (Tables 5 and 6). While some correlations were treatment-dependent (Table 5), others remained inherently significant regardless of factors (Table 6). When accounting for all experimental factors (Table 5), the concentrations of Lactic Acid (LA), Acetic Acid (AA), Propionic Acid (PA), and Butyric Acid (BA) were found to be positively correlated with each ther, but inversely related to pH. Additionally, pH levels exhibited a positive correlation with Spoilage Flora (SP) counts.An increase in AA levels is accompanied by an increase in PA and BA levels. The PA level is positively influenced by the BA level. Therefore, an increase in AA levels results in a decrease in the number of Lactic Acid Bacteria (LAB) and SP. As for PA levels, an increase leads to an increase in BA and a decrease in the number of SP. An increase in the number of LAB leads to a decrease in BA levels.

The other relationships described previously between some parameters are naturally significant. Indeed, analyzing the relationships by controlling for the effects of all factors (Table 6) shows that the influence of AA, PA, and BA levels on the pH drop is dependent on the factors studied (Variety, Cutting Stage, Salt Addition). Only the increase in LA levels is naturally linked to a pH drop. Significant relationships, masked by the effects of the factors (Table 5), appear between ammonia levels and those of acetic and propionic acids (Table 6). These relationships show that these three factors move in the same direction. The significant relationships between LA levels and those of PA and BA are dependent on the effects of the factors. In contrast, the relationship between LA and AA is naturally significant. Also, the significant negative relationships between the number of SF and the levels of AA and PA are dependent on the factors. These parameters naturally depend on the levels of PA and BA (positively) and the number of LAB (negatively) with regard to AA, and on BA (positively) with regard to PA. As for BA, it is naturally influenced by the number of LAB (negatively).

Table 5 Correlation matrix between organic acid content and microbiological parameters of silage, with the effects of variety, harvest stage, and salt addition

Parameters	pH	NH3 (%)	LA (%)	AA (%)	PA (%)	BA (%)	BAB (log CFU/g)	LAB (log CFU/g)	SF (log CFU/g)
pH	1								
NH3 (%)	0.111	1							
LA (%)	**-0.426** **	0.094	1						
AA (%)	**-0.629** **	0.173	**0.368** **	1					
PA (%)	**-0.455** **	0.220	**0.333** *	**0.645****	1				
AB (%)	**-0.615****	0.021	**0.367****	**0.827****	**0.846****	1			
BAB (log CFU/g)	-0.125	-0.156	0.190	0.067	0.023	0.078	1		
LAB (log CFU/g)	0.230	0.152	-0.199	**-0.268** *	-0.217	**-0.317** *	-0.144	1	
SF (log CFU/g)	**0.344** **	-0.118	-0.067	**-0.320** *	**-0.320** *	-0.258	0.200	0.041	1

Legend: NH3 – Ammonia, **LA** – Lactic Acid, **AA** – Acetic Acid, **PA** – Propionic Acid, **BA** – Butyric Acid, **BAB** – Butyric Acid Bacteria, **LAB** – Lactic Acid Bacteria, **SF** – Spoilage Flora, **CFU** – Colony Forming Units, *** – The correlation is significant at the 0.001 level, ** – The correlation is significant at the 0.01 level, * – The correlation is significant at the 0.05 level.

Table 6 Correlation matrix between organic acid content and microbiological parameters of silage, without the effects of variety, harvest stage, and salt addition

Parameters	pH	NH3 (%)	LA (%)	AA (%)	PA (%)	BA (%)	BAB (log CFU/g)	LAB (log CFU/g)	SF (log CFU/g)
pH	1								
NH3 (%)	0.122	1							
LA (%)	**-0,364****	0.123	1						
AA (%)	-0.258	**0.289***	**0.279***	1					
PA (%)	-0.106	**0.334***	0.178	**0.501*****	1				
BA (%)	-0,247	0,106	0,177	**0,781*****	**0,731*****	1			
BAB (log CFU/g)	-0,103	-0,155	0,097	-0,042	-0,071	-0,107	1		
LAB (log CFU/g)	0,245	0,147	-0,173	**-0,304***	-0,216	**-0,413****	-0,117	1	
SF (log CFU/g)	0.182	-0.133	-0.011	-0.152	-0.216	-0.093	0.211	0.026	1

Legend: NH3 – Ammonia, **LA** – Lactic Acid, **AA** – Acetic Acid, **PA** – Propionic Acid, **BA** – Butyric Acid, **BAB** – Butyric Acid Bacteria, **LAB** – Lactic Acid Bacteria, **SF** – Spoilage Flora, **CFU** – Colony Forming Units, *** – The correlation is significant at the 0.001 level, ** – The correlation is significant at the 0.01 level, * – The correlation is significant at the 0.05 level.

DISCUSSION

Introduction to Pearl Millet Fermentation Quality

The results confirm the potential of pearl millet (*Pennisetum glaucum*) for silage production, with very satisfactory average pH levels, particularly at the flowering stage where they fall below 4.05 (Mean HS, Table 1). This rapid acidification is essential to inhibit undesirable microorganisms and stabilize the forage. Studies conducted in sub-Saharan Africa on millet silage have also reported satisfactory fermentation profiles, with a dominance of lactic acid bacteria after ensiling and good nutrient preservation **(Cai *et al.*, 2020; Korombé *et al.*, 2023a; Korombé, 2024)**. However, the analysis reveals significant variability in fermentation and microbiological parameters depending on the treatments applied. The objective of this trial was to identify the optimal management conditions (variety selection, harvest stage, salt addition) to maximize acidification and ensure aerobic stability. The following discussion aims to analyze the influence of these factors and their interactions to propose a technical approach to ensure reliable millet silage production.

The Predominant Influence of Variety

The effect of "Variety" is highly significant ($p < 0.001$) on the final pH (average variety). The varieties Chakti (pH 4.30), Alambana (4.25), and ICRI-Tabi (4.31) have low average pH values, while Siaka Millet (4.03) stands out with the lowest pH, indicating excellent fermentation potential. Conversely, the local variety Sadoré (4.15) and especially Maywa (4.26) show slightly less efficient acidification.

These varieties differed in their earliness, with Chakti, Mil de Siaka, Icri -Tabi, and Alambana being early-maturing improved varieties, and Locale Sadoré and Maywa being later-maturing local varieties. It is generally accepted that the earliness of a plant strongly influences its soluble sugar content, which is usually higher in early-maturing varieties due to accelerated metabolism and rapid accumulation before end-of-cycle stresses. Furthermore, efficient photosynthesis, characteristic of early-maturing plants, promotes the production of sugars (glucose, sucrose) that act as osmoregulators, increasing tolerance to stress (drought, cold) **(Drossopoulos) *et al.*, 1987; Rodrigues *et al.*, 2010; Ouazzani and Moustaghfir , 2020)**. This would undoubtedly be linked to periods of drought recorded during the production of the residues used in our study.

Furthermore, it is also established that the soluble carbohydrate content of forages directly influences the fermentation characteristics and quality of silage **(McDonald *et al.*, 1991; Rooke and Hatfield, 2003; AFSSA, 2004; Baumont *et al.*, 2011; Kang *et al.*, 2018)**. Varieties such as Siaka Millet and Chakti likely possess a higher concentration of fermentable sugars, thus providing an abundant substrate for lactic acid bacteria (LAB) to produce lactic acid (LA) and lower the pH **(Korombé) *et al.*, 2023a; Korombé, 2024)**.

The Maywa variety exhibits an atypical fermentation profile. It displays the highest average levels of acetic acid (AA: 1.91%), propionic acid (PA: 1.64%), and butyric acid (BA: 2.03%), as well as ammonia (NH3: 0.05%). These compounds are indicators of undesirable secondary fermentations. The accumulation of AA and PA can signal heterofermentative activity, but the significant presence of BA and NH3 is the signature of clostridial fermentation **(Van Os, 1997; Ward, 2000; Seglar, 2003; Amuda, 2013)**. This proteolytic degradation with the formation of NH3 and carbohydrates degradation into butyric acid suggests that the Maywa variety possesses intrinsic characteristics that make it more vulnerable, such as a high buffering capacity linked to a higher protein or mineral content, as reported by **Korombé *et al.* (2023b)** or a higher water content, which would have slowed the initial drop in pH and allowed the germination of butyric spores. Bacteria of the genus *Clostridium* are indeed ubiquitous contaminants of soil and forage, capable of fermenting sugars, lactic acid or proteins to produce butyric acid and ammonia **(Pahlow *et al.*, 2003; Driehuis, 2013)**.

The Effect of Harvest Stage

The harvest stage significantly influences pH ($p < 0.001$). The average pH at flowering (4.05) is lower than that at maturity (4.39). At flowering, the plant is still rich in soluble sugars and water, which promotes rapid and intense lactic acid fermentation. Furthermore, the plant's structure at this stage allows for better compaction during ensiling, limiting the presence of residual oxygen and thus rapidly inhibiting undesirable microflora while enhancing the activity of lactic Acid Bacteria (LAB). At maturity, the plant lignifies; its water and fermentable sugar content decreases, while complex fibers, less directly accessible to LAB, slow down acidification. Similar observations on millet residues have shown an increase in dry matter and fiber, and a decrease in protein and energy content with plant age, which necessarily affects silage quality **(Khan *et al* ., 2011; Cai *et al* ., 2020; Korombé *et al.*, 2023b)**.

Average organic acid concentrations decrease overall at the maturity stage, from 1.71% to 1.13% for AA, from 1.36% to 0.88% for PA, and from 1.93% to 0.93% for BA. This general decrease can be attributed to lower overall microbial activity at the maturity stage, due to reduced availability of readily fermentable substrates and lower water activity (**Kibe *et al.*, 1997; Ferrero *et al.*, 2019; Carvalho-Estrada *et al.*, 2020)**. It is also accepted that the composition of the epiphytic microflora evolves with the physiological stage of the plant **(Mougel *et al.*, 2006; Carvalho- Estrada *et al.*, 2020)** shifting from a flora dominated by LAB to a more diverse but less active flora, or to microorganisms more specialized in the degradation of complex substrates, producing fewer volatile organic acids. Furthermore, **Cai *et al.* (2020)** reported that after prolonged field exposure to millet and sorghum residues, aerobic bacteria dominated while LAB became undetectable.

The Role of Salt (NaCl) in Fermentation Modulation

Adding salt has a significant effect on pH, but only in interaction with the harvest stage ($p < 0.001$ for AS x HS). At flowering, salt lowers the pH (3.90 with salt vs. 4.05 without). This is explained by the osmotic effect of NaCl, which creates an environment unfavorable to many undesirable bacteria (particularly enterobacteria, which are often less halotolerant), while generally being well tolerated by lactic acid bacteria **(Muck *et al.*, 2018)**. By reducing competition for substrates, NaCl allows LAB to dominate fermentation more rapidly and produce more lactic acid **(Pahlow *et al.*, 2003)**. Moreover, there is a trend towards an increase in PA and BA with salt at flowering ($p=0.052$), which could indicate that salt, by inhibiting part of the flora, modifies the competitive balances within the acid producers themselves.

The effect of salt disappears completely (pH of 4.39 in both cases) at maturity. The most likely hypothesis is that the low water content and reduced substrate availability at this stage already constitute a major limiting factor for microbial activity. The additional osmotic effect of salt then becomes negligible compared to these pre-existing physicochemical constraints. The limiting factor is no longer microbial competition, but the resource itself. It is also reported that butyric acid bacteria (BAB), for example, require high water activity to develop **(Silvetti and Brasca, 2018)**.

Furthermore, salt concentrations above 1% would likely not be necessary to significantly suppress butyric acid bacteria under the conditions of this study. The work of **Shockey and Borger (1991)** demonstrates that a concentration of 100 mM NaCl (equivalent to approximately 0.4 to 0.5% of fresh weight, depending on water content) is sufficient to completely inhibit *Clostridium butyricum* in vitro, a threshold already reached in the trials presented. Beyond this concentration, the beneficial effect does not increase, and there is even a risk of inhibiting lactic acid bacteria, compromising the acidification necessary for preservation **(Shockey and Borger, 1991)**. In this context, strategies to improve fermentation quality should instead combine salt with specific lactic acid bacteria inoculants, whose superior efficacy has been demonstrated **(Shockey and Borger, 1991)**, rather than limiting themselves to increasing salinity.

Biological significance of three-factor interactions (Variety × Stage × Salt)

The effect of salt is not uniform. It depends on the harvest stage and the variety. Research by **Shockey and Borger (1991)** on alfalfa silage established that adding NaCl at a concentration of 100 mM (0.41 to 0.47% of fresh matter, depending on moisture content) selectively inhibits *Clostridium butyricum* while promoting salt-tolerant LAB populations. At the flowering stage, the forage is richer in sugars and less buffered, which makes the effect of salt more pronounced. In contrast, at maturity, the more woody forage provides a less fermentable substrate, as described by **Miron *et al.* (2006)**, explaining the variability in responses across varieties. **Jiang *et al.* (2024)** further confirm that different plant varieties recruit distinct rhizosphere microbial communities, which contributes to their varying silage potentials. These interactions confirm that the efficacy of an additive depends on initial conditions, both genetic and physiological.

Microbiological Dynamics and Interactions

Table 5 highlights significant negative correlations between Spoilage Flora (SF) and acetic (-0.320*) and propionic (-0.320*) acids. This suggests that these acids, produced by some bacteria (including heterofermentative LAB), play a role in inhibiting yeasts and molds. Conversely, the positive correlation between SF and pH (0.344**) indicates that when acidification is insufficient (higher pH), SF proliferates more readily. This illustrates the fundamental principle of silage preservation: rapid and strong acidification is key to locking in the ecosystem and preventing the development of spoilage microorganisms. The use of bacterial inoculants can also improve the aerobic stability of millet silage **(Camilo *et al.*, 2023)**.

Butyric acid bacteria (BAB) were present in almost all samples (Table 2), with no significant effect of the treatment. However, butyric acid (BA) production varied considerably, particularly with the Maywa variety, which accumulated a large amount (Table 1). This discrepancy is explained by the key role of pH. *Clostridia* are spore-forming bacteria found ubiquitously in soil and forage **(Driehuis, 2013)**. Their spores germinate and become metabolically active only when the pH is too high (generally > 4.5). In well-made silage (pH < 4.2), they remain dormant spores and do not produce BA **(Wieringa, 1958**; **Flythe and Russell, 2004)**. An earlier study even reported a negative correlation between silage pH and *Clostridium* spore number, suggesting that acidic conditions limit their development **(Ohba *et***

***al.*, 2002)**. Thus, the presence of BAB (log CFU/g) in the analyses does not predict their activity. The high BA levels in Maywa silage are therefore the result of initial acidification that was too slow or insufficient in this variety (pH 4.26 on average), which allowed spore germination and butyric fermentation. Recent work also confirms that pH is a critical factor, with, for example, an optimal pH of 5.5 for the growth of *C. butyricum* and 6.0 for its sporulation **(Wang *et al.*, 2024)**.
However, the use of functional categories (LAB, BAB, and spoilage flora) without species-level identification obscures the metabolic diversity that is essential for understanding these processes. **McAllister *et al.* (2018)** emphasize that molecular identification (16S sequencing, metagenomics) is indispensable for characterizing microbial interaction networks and identifying functionally key species, particularly to distinguish between homo- and heterofermentative LAB with opposing roles in acidification and aerobic stability. Furthermore, the distinction between proteolytic and saccharolytic *Clostridium* species is crucial, as only the former are responsible for protein degradation and ammonia production. The lack of a significant correlation between BAB counts and butyric acid content illustrates the gap between sporulation and actual fermentative activity. The application of modern molecular methods, as recommended by **McAllister *et al.* (2018)**, would help elucidate the fine ecological mechanisms and optimize varietal silage strategies.

Correlation Analysis

Table 6 confirms that lactic acid is the main contributor to the pH decrease. The correlation between lactic acid (LA) and pH is negative, moderate, and highly significant (-0.426**).** In contrast, the other acids (AA, PA, BA) show even stronger negative correlations with pH (-0.629, -0.455, -0.615). This does not mean that they acidify more effectively, but rather that they appear as a consequence of poor fermentation. In silage where the pH does not decrease quickly enough due to LA, secondary fermentations (heterolactic, clostridial) occur, producing these acids and contributing to a further decrease in pH, but of lower quality because it is associated with dry matter losses and risks to the animal.
The extremely strong correlations between AA, PA, and BA (from 0.645** to 0.846**, Table 5) indicate that they are produced under similar conditions, typically secondary fermentations. The positive correlation, although not significant in this table, between NH3 and PA (0.220) or AA (0.173) points to a combined process of sugar degradation (producing acids) and protein degradation (producing NH3). This signature is characteristic of clostridial activity, where *Clostridium* ferments both carbohydrates (into BA, AA) and amino acids (into NH3, AA, PA) via the Stickland reaction **(Driehuis, 2013)**. This confirms that the appearance of NH3 is a reliable indicator of undesirable proteolysis, often coupled with volatile acid production in a context of primary lactic fermentation failure.
The successful acidification of silage despite low concentrations of lactic acid bacteria (LAB)—as evidenced by the absence of a significant positive correlation between LAB concentration and pH—can be explained by the typical microbial succession observed in silage. Conventional culture techniques provide only a partial picture of microbial diversity, underestimating the true complexity of the communities involved. Fermentation follows a three-phase dynamic: an initial phase of rapid proliferation of epiphytic LAB (up to 10^8 –10^9 CFU/g), followed by a phase dominated by acid-tolerant populations, and then a natural decline toward a stable phase where bacteria enter a VBNC (viable but non-cultivable) state, undetectable by standard culture methods, which require cells in active growth. This viable but non-cultivable state may be induced by starvation, drying, radiation and toxic peroxides produced by the LAB themselves during aerobic growth **(Condon, 1987; Muck, 1988; Pahlow *et al.*, 2003; McAllister *et al.*, 2018)**. Therefore, the low concentrations of lactic acid bacteria in our silage may be the result of the depletion of soluble sugars, which serve as food for these bacteria. This is further supported by the correlations between acetic, propionic, and butyric acids observed in the results, which stem from a dominant heterofermentative metabolism typical of substrates low in water-soluble carbohydrates (WSC) or with high buffering capacity, as confirmed by **Miron *et al.* (2006)** in their study on forage sorghum varieties.

CONCLUSION

This study demonstrates that the fermentation quality of millet residue silage in Niger is significantly influenced by variety, harvest stage, and salt addition. The results indicate that the harvest stage is the primary determining factor. Although it results in a slightly higher pH, harvesting at maturity yields silage with significantly lower levels of butyric and propionic acids key indicators of poor fermentation. Consequently, this stage is the most effective for minimizing the spoilage risks. The choice of variety also appears to be crucial. Genotypes such as Siaka Millet stand out due to their lower pH and more stable fermentation profiles, likely owing to intrinsic characteristics (such as sugar content, buffering capacity) that favor preservation. Additionally, incorporating 1% salt exerts a positive modulating effect, specifically by lowering the overall pH and stimulating acetic acid production, which in turn enhances aerobic stability.
Although its effects on microbial populations were not statistically significant, the observed trend toward a reduction in butyric acid bacteria and spoilage flora is encouraging.
In practice, to optimize millet residue silage under conditions similar to those in Niger, it is recommended to: i) prioritize harvesting residues at the full grain maturity stage, ii) select suitable varieties such as Siaka Millet, and iii**)** incorporate 1% salt as a simple, affordable, and effective additive to improve acidification and preservation quality. This combination of practices offers an accessible technical solution for utilizing this abundant resource and improving feed security in the Sahel region.
However, this study must be continued in order to identify the different strains of microorganisms and gain a better understanding of the fermentation profiles of the silages studied. One of the main limitations lies in the use of conventional cultural methods for microbial enumeration, which neither allow discrimination of metabolic pathways (homofermentative vs. heterofermentative) within lactic acid bacteria, nor distinguish between functionally active species and dormant spore-forming forms. The absence of molecular identification (16S sequencing, metagenomics) thus limits the understanding of the fine ecological mechanisms underlying the observed variety × stage × salt interactions. Complementary analyses using molecular biology would be necessary to characterize the dynamics of microbial communities and optimize variety-specific silage strategies.

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