Effect of Spices on the Microbial Diversity, Physicochemical Properties and Nutritional Properties of Fermented Millet (*Pennisetum glaucum*) Slurry

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ABSTRACT

Fermentation improves taste, adds flavour and makes nutrients easily digestible. Millet porridge is produced from spontaneous fermentation of pearl millet grain with or without spices. A study was carried out to evaluate the proximate, nutritional, and bacterial diversity composition during fermentation of pearl millet slurry with and without spices. In this experimental study, some millet grains were fermented with selected spices, while others were fermented without spices. The pH, proximate and mineral analysis, and lactic acid production were determined in the fermented products. The 16S ribosomal RNA (16S rRNA) metagenomic method was used to identify the microbial diversity and abundance in the fermented millet slurry with and without spices. The slurry fermented with spices recorded 22.50 (mg/g) iron at 24 hours whilst without spices recorded a 10.10 (mg/g) iron content. Furthermore, zinc content at 24 hours for slurry with spices was 175.50 (µg/g) whilst without spices was 60.10 (µg/g). The lactic acid content for millet slurry without spices was between 7.16µg/mL and 9.22µg/mL whilst the lactic acid content for millet slurry with spices was between 6.55µg/mL and 9.88µg/mL produced after a 72-h fermentation period. Acetobacter was the most dominant genera in the fermented slurry (54.23%). The relative abundance of the genus Lacticaseibacillus (19.71% to 1.69%), Lactobacillus (0.49% to 5.25%), Limosilactobacillus (2.43% to 40.96%), Acetobacter (54.23% to 34.93%), Schleiferilactobacillus (16.29% to 0.84%) were present in the fermented slurry. The fermentation of pearl millet grains with spices improves the nutritional composition of pearl millet and provides a diversified fermenting bacteria community. The most dominant species in the slurry fermentation can be formulated into starter cultures to be used in controlled fermentation.

Keywords: Bacteria Diversity, Fermentation, Millet, Lactic Acid, Nutrition, Spices

I. INTRODUCTION

Pearl millet (*Pennisetum glaucum*) is the most widely grown type of millet. It is mostly grown in arid and semi-arid regions of Africa and Asia, providing a source of nutrition for millions of people. Pearl millet is known for its ability to thrive in harsh environmental conditions, making it a valuable food source in regions prone to droughts and other environmental conditions (Serba et al., 2020). Pearl millet is mostly consumed as flatbreads, porridge, and alcoholic beverages. Additionally, the crop is used as animal feed, and its dried stalks and leaves are an important source of livestock fodder (Tonapi et al., 2024). In Ghana, the grain is fermented with the addition of spices to prepare food such as “koko”, “koko sour water”, and a local beverage popularly known as “zonkom” which is mostly consumed by people.

Millet is nutritionally significant, providing antioxidants like flavonoids, myricetin, and lignins that enhance nutrient bioavailability through enzymatic activity (Saini et al., 2021). The grain is a rich source of carbohydrates, proteins, fats, dietary fiber, and essential micronutrients, including zinc, iron, calcium, manganese, phosphorus, and magnesium (Kambabazi et al., 2019; Kumar et al., 2021). Millet consumption has been linked to cancer prevention, cardiovascular health, tumor suppression, blood pressure regulation, and improved gastrointestinal health (Saleh et al., 2013). Due to its high protein content, it also helps combat malnutrition, making it an ideal food for pregnant women, children, and individuals with diabetes (Manjula et al., 2015). As a gluten-free grain, it offers a valuable dietary alternative for individuals with celiac disease and gluten intolerance, contributing to gut health with its low glycemic index, ease of digestion, and anti-inflammatory properties (Ramashia et al., 2019). However, millet also contains

antinutritional factors, such as tannins, phytates, polyphenols, and trypsin inhibitors. Processing methods like soaking and fermentation can reduce these compounds, improving mineral absorption and enzyme activity (Sarita & Singh, 2016).

Fermentation plays a critical role in enhancing millet's nutritional composition, flavor, and shelf life. This age-old process, which involves the breakdown of complex molecules into simpler compounds via microbial activity, improves digestibility and food preservation. Yeast enzymes, for example, convert sugars and starches into alcohol, while proteins degrade into peptides or amino acids (Sharma et al., 2013). The fermentation of millet promotes the growth of beneficial microorganisms, such as lactic acid bacteria (LAB), which increase acidity, improve texture, inhibit harmful microbes and enhance the bioavailability of nutrients, providing antioxidant and antimicrobial benefits (Diaz et al., 2019, Kambabazi et al., 2019; Taylor & Duodu, 2014).

Understanding the microbial diversity in millet fermentation is essential for optimizing its benefits. Traditional methods of microbial identification, such as in vitro culture techniques, often fail to detect non-culturable microbes, leading to an incomplete understanding of microbial communities (Inglis & Edwards, 2022). However, metagenomics which analyzes environmental genetic material through sequencing has revolutionized the study of microbial diversity. This technique offers a precise characterization of unculturable bacteria, shedding light on microbial dynamics and the functional genes of microbes involved in millet fermentation (Singh et al., 2009; Thomas et al., 2012).

1.1 Statement of the Problem

Fermentation enhances the nutritional value of millet grains by improving their taste, flavour, and shelf life through microbial activity (Taylor & Duodu, 2014). Most research reports traditional culturing methods in microbial community characterisation and little is known of the impact of spices on millet fermentation (Sinclair et al., 2025). However, the specific microorganisms involved in millet fermentation have not been fully characterized using modern sequencing techniques. A deeper understanding of the microbial community is essential for optimizing fermentation processes and improving the quality of fermented millet products. 16S rRNA metagenomics provides a powerful tool for identifying and characterizing microbial populations in complex environments, including fermented foods (Klindworth et al., 2001).

1.2 Research Objectives

i. Evaluate the effect of spices on the nutritional composition of fermented millet slurry.
ii. Assess the effect of spices on the lactic acid production in fermented millet slurry.
iii. Determine the effect of spices on the bacteria diversity of fermented millet slurry.

II. LITERATURE REVIEW

2.1 Theoretical Review

Pearl millet is rich in essential nutrients, including phenolic compounds, amino acids, minerals, and micronutrients, making it superior to many other cereals in terms of energy, protein, and macronutrient content (Budhwar et al., 2020; Hassan et al., 2021). Phytochemicals such as flavonoids and polyphenols, predominantly found in the bran layer, provide antioxidant, anti-inflammatory, and antimicrobial benefits (Hassan et al., 2021; Sarita, 2016). These bioactive compounds contribute to the functional properties of fermented millet products.

Fermentation enhances the flavour, texture, and nutritional profile of pearl millet through microbial activity. It is typically a submerged process that improves the taste, flavour, and bioavailability of nutrients (Atter et al., 2021; Kambabazi et al., 2019; Taylor & Duodu, 2014). Lactic acid bacteria (LAB) dominate the process, producing organic acids that reduce pH, enhance endogenous phytase activity, and increase the solubility and absorption of key minerals such as iron and zinc (Krishnan & Meera, 2018). This transformation improves the nutritional quality of millet-based foods and extends shelf life.

Spices, derived from dehydrated plant parts, enhance the sensory quality and safety of fermented foods. Ginger (Zingiber officinale), clove (Syzygium aromaticum), negro pepper (Xylopia aethiopica), and cayenne pepper (Capsicum annuum) are commonly used for their antimicrobial, antioxidant, and bioactive properties (Borquaye et al., 2017; Lu et al., 2019). These spices influence microbial dynamics, selectively inhibiting spoilage organisms while promoting beneficial fermentative bacteria (Adisa et al., 2019). However, the extent to which they modulate bacterial community structure and fermentation efficiency in pearl millet remains understudied.

16S ribosomal RNA (16S rRNA) amplicon sequencing is an advanced method for characterizing bacterial communities involved in pearl millet fermentation. This technique enables precise identification, classification, and quantification of microbial taxa in complex biological environments (Church et al., 2020). Unlike culture-dependent

approaches, 16S rRNA sequencing captures a broader microbial spectrum, including non-cultivable species, providing insights into taxonomic composition and potential functional roles (Sinclair et al., 2015; Youseif et al., 2021).

2.2 Empirical Review

Previous studies have characterized bacterial communities in fermented pearl millet (Mishra & Sabikhi, 2020). Research indicates that LAB-dominated fermentation enhances the nutritional quality of millet-based foods. LAB produces organic acids that contribute to improved taste, texture, and increased mineral bioavailability (Krishnan & Meera, 2018). According to (Adelekan et al., 2021) spices such as turmeric and ginger enhance the nutritional profile by increasing protein, fat, and mineral content, as well as improving antioxidant properties. They further contribute to reducing anti-nutrients like phytates and tannins, which enhances the bioavailability of essential nutrients. Additionally, these spices positively influence microbial dynamics during fermentation, potentially improving the safety and shelf-life of the product. The inclusion of ginger and garlic supported lactic acid bacterial growth, reduced total bacterial and fungal counts, and contributed to the safe fermentation of ogi (Adejobi et al., 2024). Although microbial diversity in fermented pearl millet has been explored, limited research exists on how spice addition influences bacterial composition and metabolic activity. The modulation of bacterial community structure and fermentation efficiency by spices remains largely unexplored.

This study aims to address the identified gaps by investigating how selected spices affect bacterial communities and nutritional attributes in pearl millet fermentation. Employing 16S rRNA sequencing, it seeks to adequately characterise the microbial community and develop standardized protocols for producing high-quality, nutrient-rich pearl millet products. Findings will contribute to a deeper understanding of microbial ecology in spiced fermentations and support the development of functional millet-based foods with enhanced flavour, safety, and nutritional benefits.

III. METHODOLOGY

3.1 Millet Sample Collection and Preparation

Pearl millet and spices were obtained from the Cape Coast central market in the Central Region of Ghana. The spices comprised *Capsicum annum* (dried cayenne pepper), *Syzygium aromaticum* (clove buds), *Xylopia aethiopica* (negro pepper), and *Zingiber officinale* (ginger). Two fermentation assays were set-up both containing 500g pearl millet but for assay 2, 500g of ginger, 200g of dried cayenne pepper, 100g of clove buds, and 50g of negro pepper were added to the pearl millet. The samples were steeped for 24 hours, washed, and milled as prepared by vendors. The flour was mixed with five litres of distilled water and the slurry was allowed to ferment spontaneously at room temperature for 72 hr. Samples from fermented slurry were used for further analysis.

3.2 Determination of pH

The pH of the slurry fermentation in the presence and absence of spices was measured using a pH meter (TEX TEST, Microprocessor Model, Switzerland).

3.3 Determination of Proximate and Mineral Content

The proximate and mineral compositions of fermented samples were analysed following AOAC (2005) procedures. Moisture content (g/100g) was determined by drying in a vacuum oven at 105°C, fat by the Soxhlet method using petroleum ether as solvent. Protein content was analysed by the Kjeldahl method, multiplying by a factor of 6.25 for millet. The carbohydrate content was determined with the anthrone reagent. The ash content was determined by gravimetric method of incinerating samples at 550°C (Association of Official Analytical Chemist International [AOAC], 2005). The minerals iron, zinc and calcium were determined using the flame atomic absorption spectrophotometric method (AOAC, 2002). Their results were expressed in mg/100g or µg/100g.

3.4 Determination of lactic acid content

The lactic acid content of the fermented samples was analysed according to the spectrophotometric method described by Borshchevskaya et al (2016). Concentrations of lactic acid (0 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, and 0.5 mg/mL) were used as standard solutions. The fermented sample (30 mL) was diluted and centrifuged (Gallenkamp, England) at 2,000 rpm for 10 min and the supernatant was filtered off (size = 1µm, Whatman). The filtrate (2 mL) was diluted with distilled water (23 mL) and ferric chloride (2 mL) was added to a 2 mL aliquot of sample. A colour change from yellow to amber indicated the presence of lactic acid. The concentration of lactic acid was then measured at a wavelength of 390 nm using a spectrophotometer.

3.5 Metagenome DNA extraction and 16S rRNA amplification

Total genomic DNA was extracted from fermented slurry using the method described by Agyirifo et al (2019) with modification. Briefly, an aliquot (4 mL) of fermented samples were centrifuged at 14,000 rpm for 10 min. The supernatants were discarded and 300 µL extraction buffer (3.0% SDS, 1.0 M NaCl, 0.5 mM EDTA, 0.1 mM Tris-HCl, pH 8.0) and glass beads were added in the Eppendorf tubes and vortexed for 2 min. Chloroform-phenol (300µL) was added and incubated at 65 °C for 15 min. The sample was centrifuged at 14,000 rpm for 10 min and ice-cold isopropanol (500 µL) was added to the supernatant and incubated at -20 °C for 30 min. The sample was centrifuged, and the DNA was washed with ethanol (500 L) and resuspended in TE buffer (30 µL). The DNA was visualised alongside a 1 kb DNA ladder (BioLabs.) in 1% agarose gel electrophoresis stained in ethidium bromide and visualised under a UV transilluminator (Bio-Rad, USA). The metagenome 16S rRNA gene amplicon libraries were created using the Nextera XT DNA Library Preparation Kit, which utilizes V3-V4 archaeal and bacterial primers (Klindworth et al., 2012), following the manufacturer's instructions.

3.6 Illumina Sequencing

The PCR product was extracted from 2% agarose gel and purified and quantified using Herculase II Fusion DNA Polymerase Nextera XT Index V2 Kit according to the manufacturer's instructions. Purified amplicons were pooled in a paired-end sequence on an Illumina MiSeq platform (Illumina, USA) according to the standards and protocols of Macrogen Europe (Amsterdam, Netherlands). The sequence data is deposited in the NCBI database.

3.7 Bioinformatics Analysis

The paired-end reads were merged using FLASH (1.2.11). The reads were assembled, filtered and trimmed. The CD-HIT-OTU (MiSeq/FLX) program was used to pre-process and cluster the reads into the operational taxonomic unit (OUT). Clustering of Operational Taxonomic Units (OTU) was done at a cut-off of 0.03. QIIME-UCLUST was used to assign taxonomic groups. The microbial alpha diversity in slurry fermentation was determined with the Gini-Simpson index and the Shannon index.

3.8 Statistical Analysis

The experiment was conducted in triplicates and data obtained was subjected to one-way ANOVA followed by Tukey's HSD in Minitab (v 18) to assess the significant difference ($p<0.05$) among the samples. The data on proximate and mineral composition and lactic acid concentration were represented as mean values ± standard error of the mean.

IV. FINDINGS & DISCUSSION

4.1 Acidity of Fermented Slurry

The pH of the fermentation assays showed a reducing trend with no significance ($p > 0.05$) during the fermentation period (Figure 1). The pH of the millet slurry with spices (MSWS) reduced from 4.49 ± 0.29 to 3.87 ± 0.29 whilst the pH of the millet slurry without spices (MSWtS) reduced from 4.59 ± 0.3 to 3.88 ± 0.3. It was observed that the pH decreased as fermentation time increased. The decrease in pH may be due to the dissociation of large polypeptides and free amino acids that are present in the fermented slurry (Ibrahim et al., 2020; Olaniran et al., 2020). The reduction in pH may also be linked to the presence and activities of lactic acid bacteria in the medium, as well as the breakdown of carbohydrates (Sahlin & Nair, 2012). Further, a decrease in pH enhances the production of lactic acid during fermentation as the bacteria population increases (Tang et al., 2017). The increase in ash, carbohydrates, protein, and fat in the unfermented millet grains and spices could be attributed to the different spices present and their varying nutritional compositions (Borquaye et al., 2017; Samtiya et al., 2021). Though millet grains are rich in nutrients, they contain high amounts of antinutrients that form stable complexes with minerals such as calcium and zinc. The complex formed results in precipitation and makes the mineral unavailable hence could be the reason calcium and zinc after spices were added to the millet grains reduced (Borquaye et al., 2017)

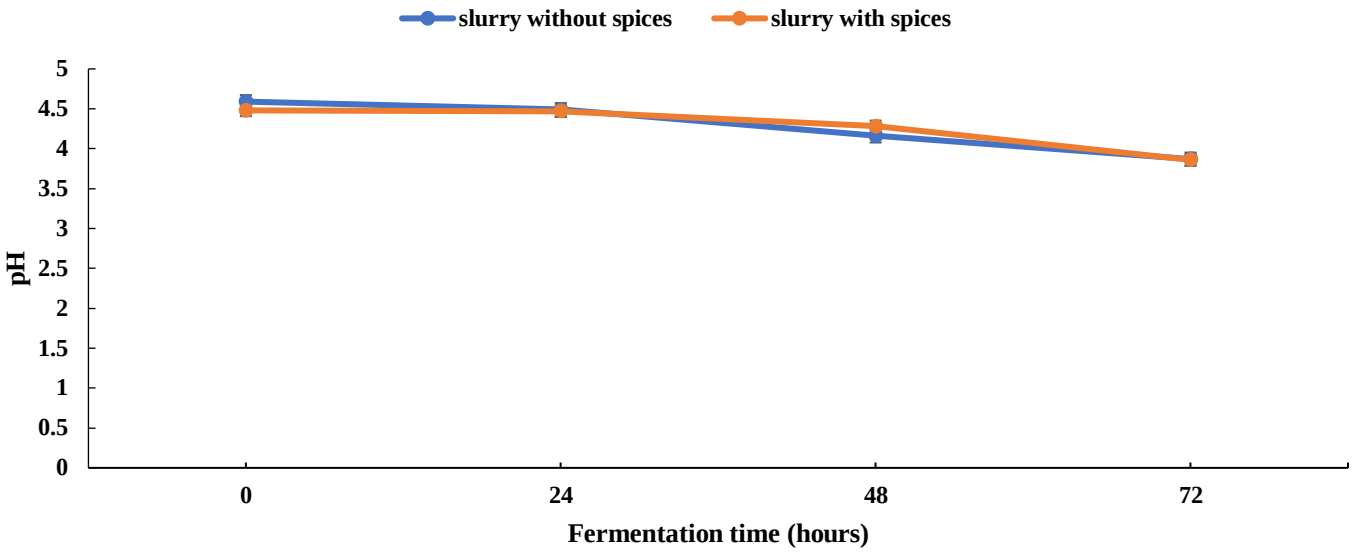

Figure 1
Changes in pH of Millet Slurry Fermented for 72 h

4.2 Proximate and Mineral Composition

In the unfermented millet grains, moisture was 15.17 g, ash 1.48 g, carbohydrate 18.34 g, protein 7.95 g, fat 4.02 g, iron 9.10 µ g/g, calcium 408.14 mg/g and zinc 110.28 µg/g. However, in the unfermented millet grains and spices, moisture was 15.39 g, ash 6.43 g, carbohydrate 63.62 g, protein 11.73 g, fat 7.62 g, iron 9.13 µg/g, calcium 345.43 mg/g and zinc 92.61 µg/g (Table 1).

Table 1
Proximate and Mineral Composition in Unfermented Millet Grains and Millet Grains and Spices

	Proximate and Mineral composition							
Sample	**Moisture (%)**	**Ash (%)**	**CHO (%)**	**Protein (%)**	**Fat (%)**	**Iron (µg/g)**	**Calcium (mg/g)**	**Zinc (µg/g)**
Millet grains	15.17[b]	1.48[b]	18.34[b]	7.95[b]	4.02[b]	9.10[a]	408.14[b]	110.28[b]
Millet+ Spices	15.39[a]	6.43[a]	63.62[a]	11.73[a]	7.62[a]	9.13[a]	345.43[a]	92.61[a]

*CHO is carbohydrates.
*Values with the same alphabet in each column are not significantly different.

The moisture content for millet slurry without spices was between 50.71% and 64.91% whilst for millet slurry with spices the moisture content was 54.36% and 65.88% after 72 h fermentation (Table 2). The difference in moisture content was statistically significant at $p < 0.05$. The ash content for millet slurry without spices was between 0.19% and 0.22% whilst for millet slurry with spices the ash content was 0.10% to 0.12% after 72 h of fermentation (Table 2). The difference in ash content was statistically significant at $p<0.05$. The carbohydrate content for millet slurry without spices was 15.25g at 0-hour fermentation increasing to 22.88g at 72-hour fermentation whilst for millet slurry with spices was 28.63g at 0-hour fermentation increasing to 31.56g after 72 h of fermentation (Table 2). The difference in carbohydrate content was statistically significant at $p<0.05$. The protein content for millet slurry without spices was between 7.56g and 8.01g whilst for millet slurry with spices the protein content was between 6.75g and 8.16g after 72 h of fermentation (Table 2). The fat content for millet slurry without spices was between 3.97% and 6.40% whilst for millet slurry with spices the fat content was between 4.74% and 6.67% after 72 h of fermentation (Table 2).

The iron content for millet slurry with spices was between 11.81µg/g and 24.92µg/g whilst for millet slurry without spices the iron content was 10.10 µg/g and 14.77µg/g after 72 h of fermentation (Table 2). The calcium content for millet slurry without spices was between 495.80mg/g and 562.90mg/g whilst for millet slurry with spices the calcium content was between 404.44mg/g and 569.28mg/g after 72 h of fermentation (Table 2). The difference in calcium content was statistically not significant at $p>0.05$. The zinc content for millet slurry with spices was between 128.93µg/g and 187.33µg/g whilst for millet slurry without spices the zinc content was between 52.20µg/g and 111.51µg/g after 72 h of fermentation (Table 2). The difference in zinc content was statistically significant at $p<0.05$.

Table 2

Proximate and Mineral Composition of Slurry Fermentation with and without Spices

Time (hour)	Sample	Proximate Composition					Mineral Composition		
		Moisture (%)	Ash (%)	Protein (g)	CHO (g)	Fat (%)	Iron (mg/g)	Calcium (mg/g)	Zinc (µg/g)
0	**MSWtS**	50.71±9.98^{a}	0.19±0.01^{a}	7.56±0.55^{a}	15.25±1.05^{b}	3.97±0.02^{d}	10.10±1.44^{b}	495.42±128.02^{a}	52.20±13.31^{b}
	MSWS	54.36±3.30^{a}	0.07±0.10^{a}	6.75±0.21^{b}	28.63±3.18^{a}	4.74±0.04^{b}	11.81±1.34^{b}	404.44±48.39^{a}	128.93±16.41^{a}
24	**MSWtS**	60.48±8.54^{a}	0.18±0.00^{a}	7.59±0.61^{a}	24.11±1.06^{a}	4.52±0.01^{c}	10.12±0.97^{b}	323.51±68.18^{a}	60.10±5.26^{ab}
	MSWS	59.64±5.44^{a}	0.13±0.09^{a}	7.96±0.54^{a}	29.87±1.88^{a}	4.35±0.05^{c}	22.50±0.98^{a}	495.43±128.40^{a}	175.50±21.68^{a}
48	**MSWtS**	62.24±3.74^{a}	0.18±0.01^{a}	7.90±0.36^{a}	22.62±1.49^{a}	6.46±0.04^{a}	12.31±2.41^{ab}	449.65±179.42^{a}	74.00±31.45^{ab}
	MSWS	60.07±3.49^{a}	0.10±0.04^{a}	7.97±0.54^{a}	31.26±1.77^{a}	6.38±0.00^{a}	23.54±1.60^{a}	561.89±28.09^{a}	182.71±9.73^{a}
72	**MSWtS**	64.91±2.21^{a}	0.22±0.06^{a}	8.01±0.07^{a}	22.88±0.37^{a}	6.40±0.00^{b}	14.77±0.72^{a}	562.90±54.02^{a}	111.51±22.04^{a}
	MSWS	65.88±5.21^{a}	0.12±0.01^{a}	8.16±0.05^{a}	31.56±1.22^{a}	6.67±0.23^{a}	24.92±3.76^{a}	569.28±23.06^{a}	187.33±75.15^{a}

***MSWtS** = millet slurry without spices, M**SWS** = millet slurry with spices, **CHO** = carbohydrates
*Values with the same alphabet in each column are not significantly different.

4.3 Lactic and Acetic Acid Produced in Slurry Fermentation with and without Spices

The lactic acid content for millet slurry fermented without spices increased from 7.16µg/mL to 9.43µg/mL whilst for slurry fermented with spices the lactic acid content increased from 6.55µg/mL to 9.62µg/mL from 0h to 72h fermentation. The difference in lactic acid content was statistically significant at $p<0.05$ (Table 3). Generally, the acetic acid content produced in the slurry fermented with spices was higher than the slurry without spices (Table 3). The increase in lactic acid and acetic acid content may be due to the metabolic activity of *Lactobacillus* species present. The presences of these acids further inhibit the growth of other microorganisms (Zhao et al., 2021).

Table 3

Lactic and Acetic Acid Produced in Slurry Fermentation with and without Spices

Time (hour)	Sample	Lactic acid (µg/mL)	Acetic acid (µg/mL)
0	**MSWtS**	7.16±0.04	6.70±0.14
	MSWS	6.55±0.22	6.52±0.78
24	**MSWtS**	7.20±0.08	6.81±0.09
	MSWS	9.03±0.21	7.21±0.25
48	**MSWtS**	7.54±0.14	7.42±0.16
	MSWS	9.47±0.10	7.71±0.31
72	**MSWtS**	9.43±0.24	9.11±0.00
	MSWS	9.62±0.21	9.78±0.42

MSWtS = Slurry without spices **MSWS =** Slurry with spices

The high moisture content noticed in the fermented slurry with spices may be due to decrease in dry matter content and the generation of certain organic acids during fermentation. (Assohoun et al., 2013; Mutshinyani et al., 2020). However, a decrease in moisture content in the millet grains without spices from 24 hours to 48 hours relates to the conducive environmental conditions provided for microbial growth that cause the moisture to decrease (Desta et al., 2021). An increase in ash content in the fermented slurry with spices and without spices could be attributed to the loss of dry matter and other volatiles and the introduction of fermenting microorganisms during fermentation (Mutshinyani et al., 2020; Onweluzo & Nwabugwu, 2009). Carbohydrate decrease in slurry without spices from 48 hours to 72 hours could be attributed to the increase in α-amylase and maltase activities that degrade starch into simple sugars as a preferred substrate for microorganisms during fermentation (Adebo et al., 2022; Nkhata et al., 2018). However, the increase in carbohydrate content in the slurry with spices could be ascribed to the termination of starch degradation by

a reduced pH that hinders the activity of amylase (Osman, 2011). Protein increase and decrease is a comparative change that happens due to the loss of dry matter because of microorganisms hydrolysing and metabolising carbohydrates and fat as an energy reserve (Inyang & Zakari, 2008; Nkhata et al., 2018). The increase in proteins could be ascribed to the dilapidation of storage proteins, increase in some extra amino acids and synthesis of new proteins. Furthermore, the breakdown of carbohydrates increases protein content as microorganisms present during fermentation utilise carbohydrates (Adebo et al., 2022). The decrease in proteins after 24-hour fermentation of the slurry without spices could be because of the decrease in crude protein as protein catabolism by fermenting microorganisms increases resulting in the loss of by-products of metabolic deamination as a carbon source (Assohoun et al., 2013). The increase and decrease in fat content are likened to results obtained by (Kharat et al., 2019) in a study of fermenting pearl millet grains. According to Adebo et al (2022) and Ocheme et al (2008) A decrease in fat content at 48 hours in millet grains without spices could be attributed to the activities of fermenting organisms that produce lipolytic enzymes to metabolise fat and leach soluble inorganic salts (Kharat et al., 2019; Ocheme & Chinma, 2008). However, an increase in fat content of the fermented millet grains and slurry has been related to the possible presence of yeast strains that produce fat in pearl millet during spontaneous fermentation (Banwo et al., 2021).

The increase in iron content is due to the loss of dry matter during fermentation as fermentative microbes disintegrate carbohydrates and protein and hydrolysing of complex matrices (Sharma et al., 2020). Furthermore, a reduction in pH increases the absorption of iron as ferric iron is converted to ferrous iron improving the iron content of the fermented slurry (Zhang et al., 2021). The decrease in calcium content observed at 24 hours of slurry fermentation without spices can be attributed to the breaking down of phytates in the presence of phytase and the activities of fermentative microorganisms that used hydrolysed elements for their metabolic activities (Gupta & Meghwal, 2021). Furthermore, the calcium increase throughout slurry fermentation with spices could be related to the fact that calcium bound to antinutrients was released from chelated complex compounds through the activities of microorganisms present during fermentation (Gupta & Meghwal, 2021; Zhang et al., 2021). The increase in zinc absorption is enhanced by the production of some low-molecular-weight organic acids such as lactic acid, butyric acid, malic acid, and formic acids that lower pH and increase the endogenous activity of phytase (Krishnan & Meera, 2018). Furthermore, an increase in zinc content could be due to the loss of dry matter during fermentation as fermentative microbes disintegrate carbohydrates and protein. The breakdown of these complex matrices releases bound zinc (Gabaza et al., 2017). The production of lactic acid was higher during fermentation in the slurry with spices than in the slurry without spices. Also, homofermentative lactic acid bacteria could be present during fermentation resulting in the breakdown of sugar to lactic acid when the pH is low and inhibits cellular metabolism and their presence increases the lactic acid produced (Abedi & Hashemi, 2020; Coelho et al., 2011).

4.4 Bacterial Diversity and Composition in Fermented Slurry

The rarefaction curve indicates there was much more diversity in species at 48 fermentation hours and 72 fermentation hours than at 24 fermentation hours as the reads lengths of sequences increased (Figure 2). This supports previous research on the microbial evolution measured during fermentation by (Zhao et al., 2021). The study further reported relatively high Shannon indices during the fermentation period about an index range of 1.5 to 3.5 and high Gini-Simpson index scores close to 1 (Figure 3). An increase in the Shannon indices and Gini-Simpson index scores at 48-hour fermentation indicates a higher bacteria diversity than at 72-hour fermentation which reported a decrease in bacteria diversity which may be due to unfavourable conditions within the slurry fermentation.

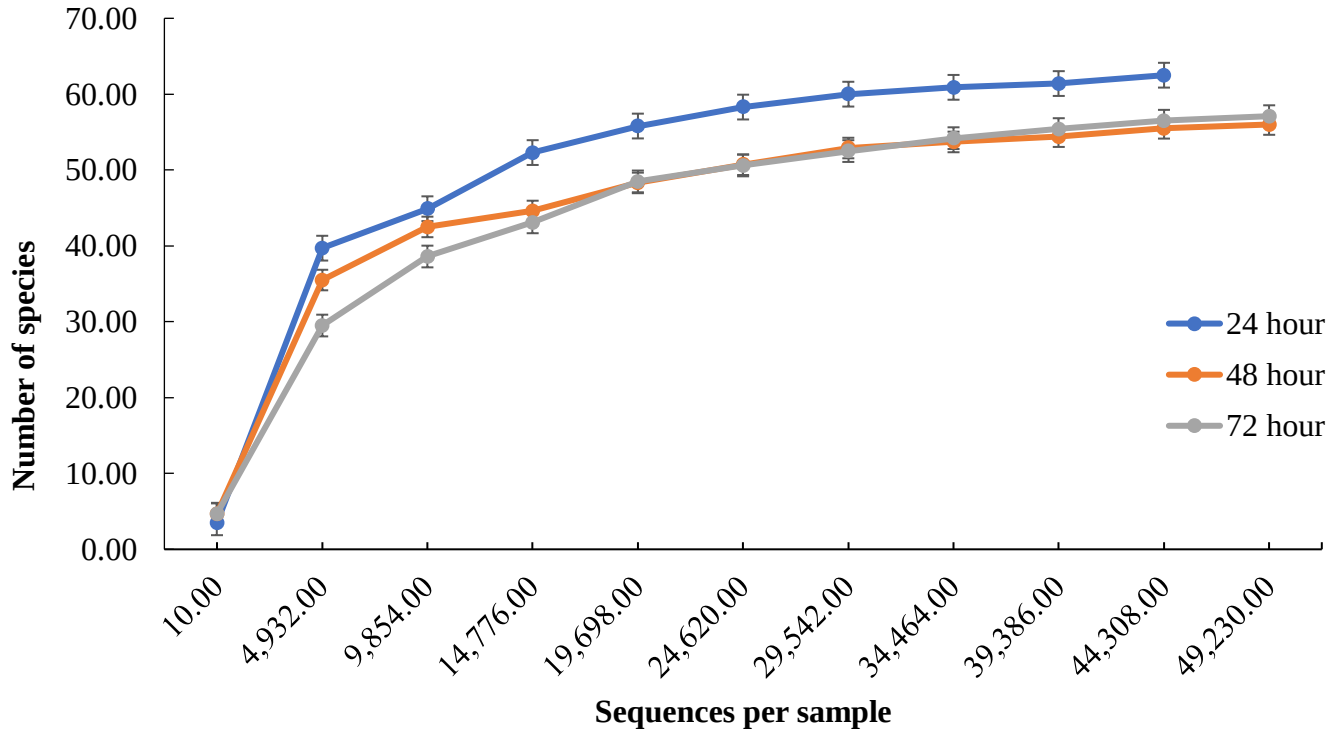

Figure 2
Alpha Diversity Showing Diversity at different Fermentation Periods

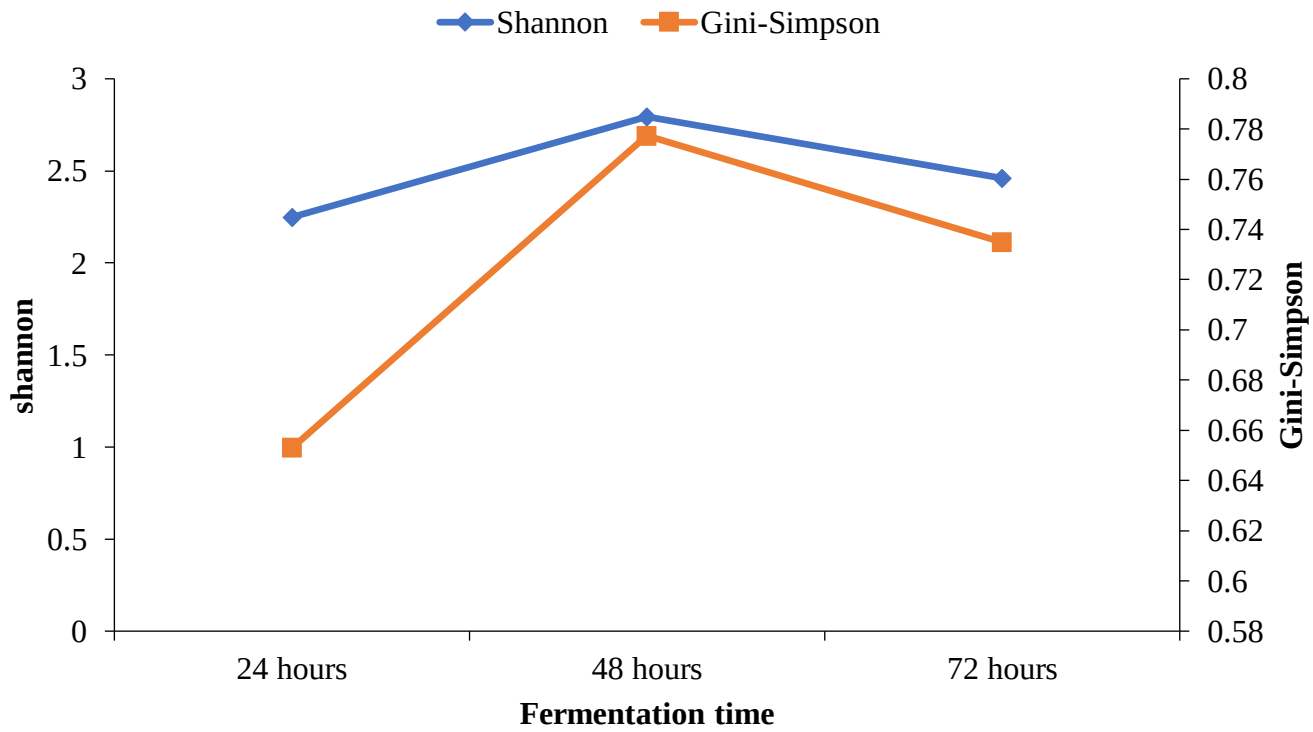

Figure 3
Trends in Identified Bacteria Diversity and Variation

4.5 Bacteria Diversity

The phyla proteobacteria was most abundant at 56.84% and Bacillota at 49.96% (Figure 4). Bacteriodota, Cyanobacteria, Actinomycetota, Verrucomicrobia and other bacteria were 0.04%, 0.01%, 0.58%, 0% and 0.04% respectively. The identified bacterial genera and their relative abundance among the fermented samples are presented in

Figure 5. The most abundant genera at 24 hours were *Acetobacter* at 54.23% and other identified genera at 24 hours were *Schleiferilactobacillus* (16.29%), *Lacticaseibacillus* (19.71%), *Limosilactobacillus* (2.43%), *Levilactobacillus* (0.90%), *Alcaligenes* (1.2%) and *Stenotrophomonas* (0.64%). Similarly, *Acetobacter* (39.21%) was the most abundant genera at 48 hours. Other identified genera at 48 hours were *Lactobacillus* (19.1%) *Limosilactobacillus* (18.07%), *Stenotrophomonas* (3%), *Lacticaseibacillus* (2.68%), *Schleiferilactobacillus* (1.79%), *Achromobacter* (1.96%), *Serratia* (0.51%), and *Weissella* (0.44%). However, Limosilactobacillus (40.96%) was the most abundant genus at 72 hours of fermentation. Other genera identified at 72 hours of fermentation were *Acetobacter* (34.93%), *Alcaligenes* (10.16%), *Lactobacillus* (5.25%), *Stenotrophomonas* (2.59%), and *Lacticaseibacillus* (1.69%).

Figure 6 shows bacteria species identified during fermentation. The most predominant species at 24 hours were *Acetobacter fabarum* (54.23%), *Schleiferilactobacillus harbinesis* (16.29%), *Lacticaseibacillus chiayiensis* (15.47%), *Limosilactobacillus fermentum* (1.27%), *Alcaligenes faecalis* (1.19%), and *Lapidilactobacillus concavus* (1.13%). Similarly, *Acetobacter fabarum* (39.2%), is seen as the most dominant species at 48 hours fermentation. Other species identified at 48 hours were *Lactobacillus crispatus* (19.4%), *Limosilactobacillus panis* (13.17%), *Limosilactobacillus fermentum* (4.03%), and *Lacticaseibacillus chiayiensis* (2.65%). The genera *Limosilactobacillus* including *Limosilactobacillus fermentum* (1.27% to 4.38%) and *Limosilactobacillus panis* (1.02% to 35.62%) and *Stenotrophomonas geniculata* (0.62% to 2.59%) were observed to increase in abundance during the fermentation period whilst *Schleiferilactobacillus harbinensis* (16.29% to 0.84%) and *Lacticaseibacillus chiayiensis* (15.47% to 1.63%) decreased in abundance during the fermentation period.

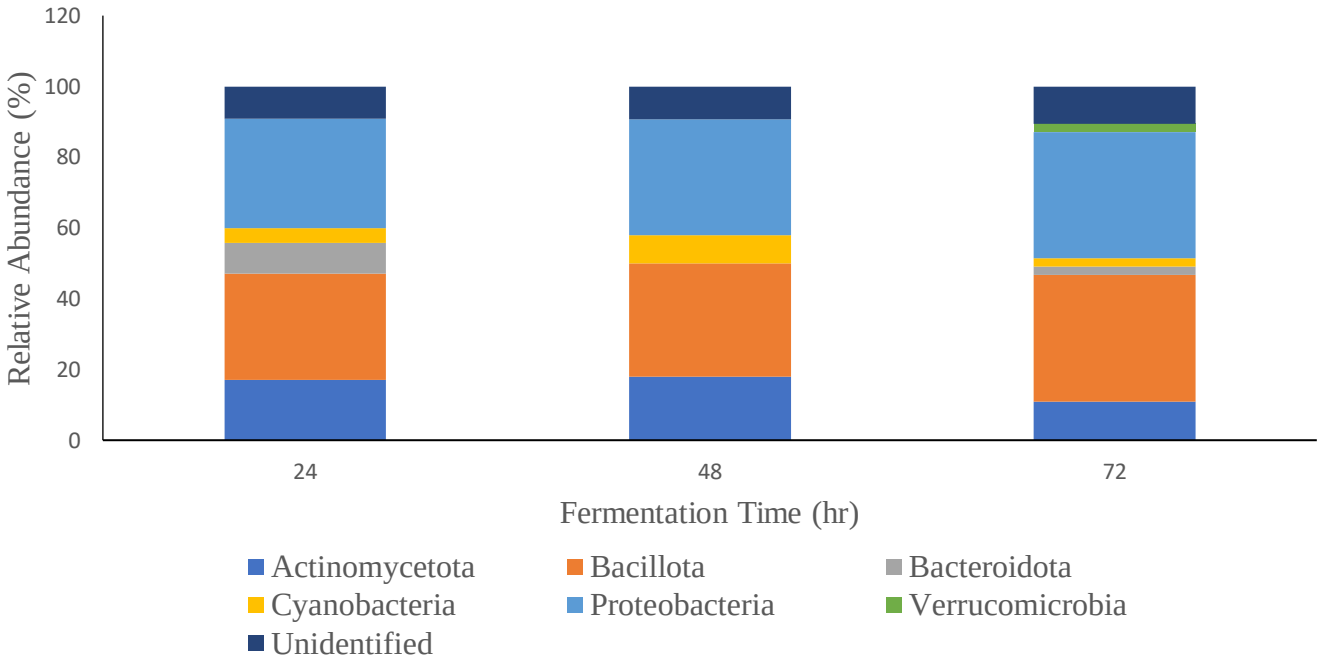

Figure 4
Relative Abundance of Bacteria Phyla associated with Millet Fermentation

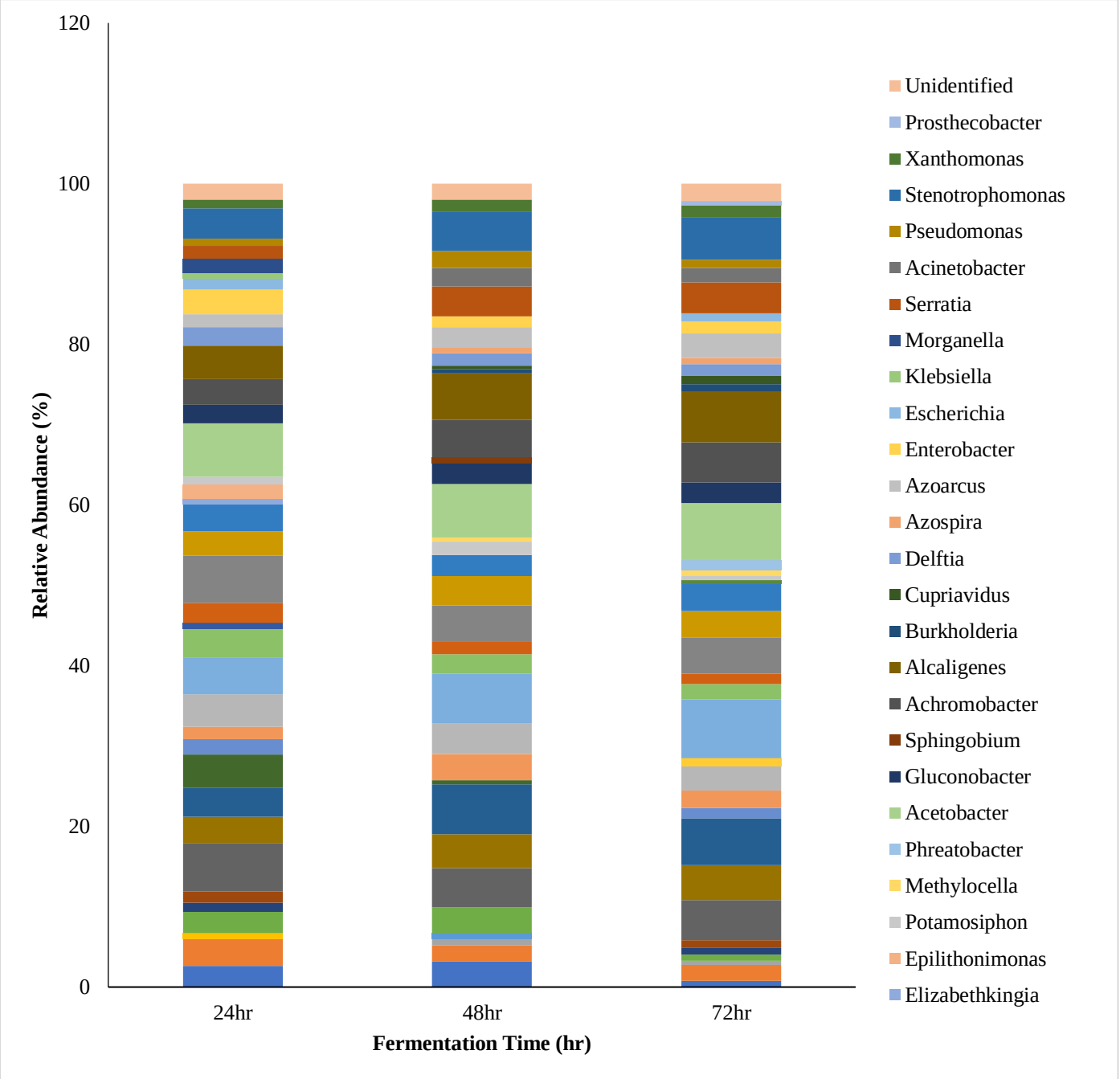

Figure 5
Relative Abundance of Bacteria Genera associated with Millet Fermentation

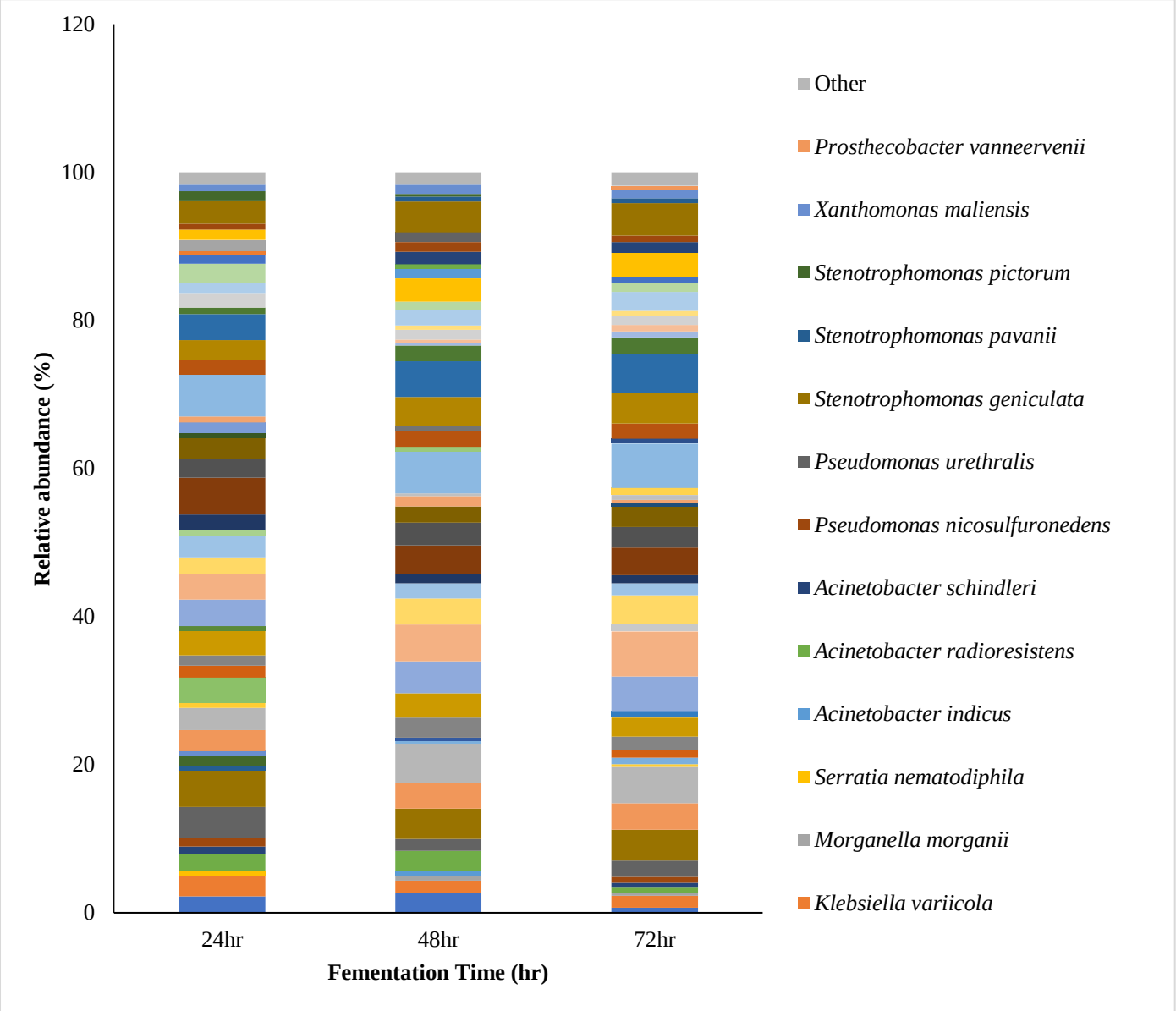

Figure 6
Relative Abundance of Bacteria Species associated with Millet Fermentation

The most abundant phyla and genera in fermented millet slurry were identified as Proteobacteria and *Lactobacillus* and *Acetobacter* respectively (Atter et al., 2021; Diaz et al., 2019). Though *Weissella* has been identified as the second most abundant genus of lactic acid bacteria present during grain fermentation (Baev et al., 2023), the current study observed a decreased population of the genus *Weisella*. This could be attributed to the fact that conditions that enhance the bacteria growth such as a higher pH are not available (Sauer et al., 2022). A decrease in pH during fermentation increases acidity and hinders the growth of most lactic acid bacteria (Wang et al., 2021).

The increase in *Limosilactobacillus, Stenotrophomonas* and *Alcaligenes* abundance from 24 hours to 72 hours indicates a favourable environment for fermentation (Li et al., 2018; Zhao et al., 2021). The decrease in abundance of *Acetobacter* genera reduced in abundance from 24 hours (54.23%) to 72 hours (34.93%) could be attributed to hydrogen peroxide produced by lactic acid bacteria that have antibiotic effects on most bacteria and the oxidation of reduced nicotinamide adenine dinucleotide (NADH) by flavin nucleotide (Abedi & Hashemi, 2020).

Lactic acid and acetic acid-producing bacteria were the most abundant species identified during slurry fermentation. The relative abundance of lactic acid bacteria species increased with fermentation time whilst the relative abundance of acetic acid bacteria species gradually decreased during fermentation. This observation was similar to the study by (Atter et al., 2021) that reported an increase in lactic acid and acetic acid-producing bacteria during millet fermentation. *Acetobacter fabarum* population decreased as the fermentation days increased because a reduced pH is not favourable to the growth of the bacteria which has a reduced acid tolerance therefore preventing its growth (El-Askri et al., 2022). Similarly, a pH lower than 5.0 increases acidity but decreases the abundance of *Lacticaseibacillus*

chiayiensis and *Schleiferilactobacillus harbinensis* as fermentation days increase. These bacteria have low acid tolerance and have their growth hindered under such conditions (Lund et al., 2020; Khan, 2014). However, some *Lactobacillus* species such as *Lactobacillus crispatus*, *Limosilactobacillus fermentum*, *Limosilactobacillus panis* and *Stenotrophomonas geniculata* a heterofermentative *Lactobacillus* species abundance increased even at low pH (Feiner, 2006).

V. CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

Spices added to millet slurry during fermentation enhance its nutritional composition and lactic acid production. Bacteria involved in millet slurry fermentation include several lactic acid and acetic acid bacteria increase as fermentation time increases.

5.2 Recommendations

It is recommended that millet should be fermented for more than twenty-four hours to allow maximum yield of metabolites. The dominant genera or species could be formulated into starter cultures and used for controlled fermentation.

Conflict of Interest

None Declared

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