The use of lactic acid bacteria as starter culture and its effect on the proximate composition and sensory acceptability of millet beverage

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Abstract

Majority of traditional cereal-based foods consumed in Africa are mostly processed by spontaneous fermentation. In Ghana, pearl millet (Pennisetum glaucum) is used for the production of fermented foods such as tuo zaafi, koko, furo-furo, masa, and fura. This study investigated some dominant lactic acid bacteria (L. brevis (S1), L. plantarum (S2), L. fermentum (S3) and L. paracasei (S4) used as starter cultures in the fermentation of millet beverage. The study further assessed the effects of the individual strains on the proximate composition and sensory characteristics of the millet beverages. The results indicate that the proximate compositions of the fermented beverages (S1, S2, S3 and S4) were significant different (p<0.05) from the unfermented beverage (UF) with the exception of moisture and fat. It was also observed that, there was significant (p<0.05) increase in protein contents in the fermented beverages with S2 having the highest % protein. The high protein content of the fermented beverages (S1, S2, S3 and S4) compared to the UF would be due to an increase in proteolytic actions during the fermentation which might have resulted in the production of essential amino acids and other simple protein compounds. With regards to the organic acids composition, the most dominant acid was the lactic acid with S2 having the highest (4.81g/l). The sensory analysis revealed that the S1 fermented millet beverage was the most preferred followed by S2. The differences in sensorial properties were significantly (p<0.05) associated with the kind of lactic acid bacteria used in the fermentation. Furthermore, the results showed that millet beverage with good color, taste and aroma was preferred. In general, S1, S2, S3 and S4 were all significantly different from UF due to the fact that fermentation is important for improving product sensory attributes and safety by reduction of toxic cyanogenic glucosides.

Keywords: Fermentation, lactic acid, sensory analysis, organic acid, principal component analysis

Introduction

Pearl millet (Pennisetum glaucum) is an important food across the Sahel region of Africa. It is a cereal commonly used for the production of many staple foods in West Africa. Pearl millet has a number of advantages including tolerance to drought, leached acid sandy soils with very low clay and low organic matter content. The grain is generally superior to sorghum as human food and at least equals maize in value as a feed grain. In Ghana, millet is used for the production of fermented foods such as tuo zaafi, koko, furo-furo, masa, and fura (Owusu-Kwarteng, Tano-Debra, Akabanda, Nielsen, & Jespersen, 2013). The processing of pearl millet to beverage involves dehulling, washing soaking of the millet grains followed by wet milling into dough which is fermented over a period of about 12 h. Majority of traditional cereal-based foods consumed in Africa are processed by spontaneous fermentation (Owusu-Kwarteng, Tano-Debra, Akabanda, & Glover, 2010). Over the years, fermentation has become part of the traditional and cultural norm among the indigenous communities in most developing countries, especially in Africa (Chelule, Mokoena, & Gqaleni, 2010). Furthermore, fermented foods constitute an important component of African diets. Many fermented foods serve as main course meals, beverages and condiments. Those which serve as main meals and beverages are usually products of carbohydrate-rich raw materials.

The use of starter culture from dominating organisms is important for the potential production of standardized millet beverage in small industrial scale, improve its acceptability, hygienic safety and microbiological
stability (Franz & Holzapfel, 2011). Among the common organisms used for fermentation, lactic acid bacterial such as, Lactobacillus plantarum and Lactobacillus brevis are among the dominant ones in Africa. Moreover, these strains were selected based on their antagonistic activity against selected pathogenic and spoilage microorganisms, production of lactic acid and diacetyl (Temitope & Taiyese). Therefore, this study was undertaken to evaluate some of the dominant lactic acid bacteria (L. brevis, L. plantarum, L. fermentum and L. paracasei) in the fermentation of millet beverage. Besides, although equipment and procedures used for millet beverage processing are relatively simple, the microbial aspects have not been adequately researched especially using single culture.

Materials and Methods

Materials

Pearl millet (Pennisetum glaucum) was purchased from a project farm in Navrongo, Ghana, DeMan, Rogosa and Sharpe (MRS) broth and MRS agar were purchased from Sigma-Aldrich (Shanghai, China), Lactobacillus (L.) plantarum (ATCC SD5209), Lactobacillus (L.) brevis (ATCC No: SD5214) were obtained from DuPont China Holding Co. Ltd (Shanghai, China). Pure organic acid standards were procured from Sigma-Aldrich (Shanghai, China). All other analytical grade chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Methods

Starter culture preparation

The lactic acid bacteria (LAB) strains were activated by culturing in Man Rogosa Sharp broth (MRS, Merck) at 35 °C for 24 h. The microbial culture was centrifuged (Kaida KL05A) at 3500 rpm for 10 min. The supernatant was discarded and microbial cells washed using 0.1% sterile NaCl solution with the aid of hemocytometer version XB-K-250 (Jianling Medical Device Co., Danyang, China).

Beverage production

The pearl millet grains were steeped in tap water at room temperature for 36 h after which grains were drained and milled in grain grinder machine (GELGOOG GG9FZ-21, Henan Gelgoog Commercial & Trading Co., Ltd, China). The beverage was constituted by dissolving the dough (15% w/v) in water. The mixture was heated at 100 °C for 1 h. Thereafter, the brix was adjusted to 20 using sucrose (based on previous study, unpublished data). After which 500 ml of the mixture was dispensed into 1000 ml Erlenmeyer flasks and inoculated with the activated LAB strain (2% v/v) and incubated at 37 °C for 48 h in a rotary incubator (IS-RDD3, Crystal Technology and Industries, Jiangsu, China) at 150 rpm. The fermented beverage (FB) was clarified by sieving using a cheesecloth. The FB was then subjected to ultrasonic sterilization at a frequency of 34 kHz for 12 min, power of 60 W and pulse duration of 10s on and 5s off (Engmann, Ma, Zhang, Yu, & Deng, 2014) and stored at -20 °C prior to analysis. The control was millet beverage treated under the same conditions but without LAB.

Proximate analysis

Moisture content was determined gravimetrically by oven-drying at 105°C, to a constant weight using Thermo Scientific Precision Compact Oven (Gravity Convection). Crude protein, crude fat and ash were determined, using the standard methods of the Association of Official Analytical Chemists (AOAC) (1990). The ash content was determined by asching the sample at 550 °C, in a Thermo Scientific Thermolyne Compact Benchtop Muffle Furnace. Crude fat was measured by the Soxhlet extraction method with petroleum ether, using the Soxhlet apparatus (SciLabware Staffordshire, UK). The crude protein content was calculated from nitrogen content, using the Kjeldahl method (Labso1 Enterprises, Delhi, India) and multiplied by a factor of 6.25 (Nitrogen to protein conversion factor). Total carbohydrate was estimated by difference. The total soluble solids and total reducing sugars were assessed using the method of Association of Official Analytical Chemists (AOAC) (1990).

Analytical methods

Determination of pH and organic acids

The pH of the fermented millet beverage was determined using a digital pH meter (LIDA Instrument PHS-3C Precision pH/mV meter). Organic acids were determined by HPLC according to the method described by Shui and Leong (2002) using HPLC system (LC-10A HPLC Series, Shimadzu, Kyoto, Japan) equipped with an Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad Labs., Richmond, CA, USA) and UV/Vis detector (SPD-20A).
Microbiological analysis

The population dynamics of the LABs were monitored at different periods (0, 6, 12, 24, 36 and 48 h) during the fermentation. In sum, a 1 in 10 dilution was prepared by aseptically transferring 1 ml of the beverage into 9 ml sterile distilled water and vortexed for 30 s. Thereafter, serial dilutions were prepared and 1 ml of an appropriate dilution pour plated using MRS agar. Plates were incubated at 37 °C for 48 h and colony forming units (CFU)/ml estimated.

Sensory assessment

The sensory assessment was conducted using 35 untrained panellists (14 males and 16 females) between the ages of 20 and 45 comprised of students and staff of the Cape Coast Technical University. Panellist were presented with 3 code samples and water to rinse their mouth after tasting each sample. Each panel evaluated samples (20 ml) for colour, aroma, taste, mouthfeel and overall acceptability on a 9-point hedonic scale (1=dislike extremely; 2= dislike very much; 3= slightly dislike; 4= dislike 5=neither like nor dislike, 6=like; 7=slightly like; 8=like very much; 9=like extremely). Their responses were recorded on a sensory sheet designed by the authors.

Statistical analysis

Treatments and analyses were carried out in triplicates and the results presented as mean ± standard deviation. The means were compared at p< 0.05 significance level using Tukey test. The analysis of variance (ANOVA) and graphical plots were performed using OriginPro version 2015 (OriginLab, Northampton, USA). Principal Components Analysis (PCA), partial least squares regression (PLSR) and principal component regression (PCR) were performed using XLSTAT 2016 software (Addinsoft, Paris, France). All the experiments were in conducted in triplicates and each experiment was repeated.

Results and discussion

The population dynamics of the lactic acid bacteria used in the fermentation were monitored from 0 h (within 1 h after inoculation into the beverage) to 48 h in the millet beverage (Fig. 1a). The findings indicated that all the strains increased slightly at the lag phase (0-6 h) and then increased sharply. From the results, it can be seen that all the strains survived and their populations increased throughout the fermentation process indicating that the organic acids produced by the individual strains did not negatively affect their survivability. From Fig. 1a, there were no significant differences (p<0.05) among S1, S3 and S4 although at 12 – 36 h, S1 was noticed to be higher compared to S3 and S4. The results indicated that the microbial population in S2 was significantly higher than all the other treatments. These growth patterns are in line with those of (Mugula, Nkko, Narvhus, & Sørhaug, 2003). Beside L. plantarum has been reported to be predominant in most lactic acid fermented foods (Di Cagno, Coda, De Angelis, & Gobbetti, 2013; Kalui, Mathara, & Kutima, 2010; Lei, Friis, & Michaelsen, 2006; Lei & Jakobsen, 2004; Muyanja, Narvhus, Treimo, & Langsrud, 2003).

With regards to the pH of the individual lactic acid fermented beverage, the results indicated that the initial pH of the beverages were 6.6 at 0 h. Upon introduction of the strains into the beverages, it was observed that the pH dropped significantly at the end of the fermentation period (48 h). This finding indicated the strains may have converted the sugars in the beverage to acids leading to the decrease in the pH. It was noticed that at 6 h and 24 h, there were significant differences (p<0.05) in pH of the individual fermented beverages among all the samples (Fig. 1b). The pH of L. plantarum fermented beverage was the lowest at the end of the period compared to the others. Similarly, Kostinek et al. (2005) reported that L. plantarum strains were shown to be better acid producers and capable of faster acid production than L. fermentum. This may probably be the reason why the pH of L. plantarum was lowest at 6, 12 and 48 h.
It was also observed that, there was significant ($P<0.05$) increase protein contents in the fermented beverages (Temitope & Taiyese) with S2 having the highest % protein. The high protein content of the fermented beverages (S1, S2, S3 and S4) compared to the UF would be due to an increase in proteolytic actions during the fermentation which might have resulted in production of essential amino acids and other simple protein compounds (El Hag, El Tinay, & Yousif, 2002; Muyanja et al., 2003; Saleh et al., 2013). Our results also show a reduction in fat content of the beverages compared to the unfermented sample. This could be due to utilisation of the lipids by the microorganisms. Our findings supports those of Saleh et al. (2013).

**Organic acid composition**

The organic acids composition of the samples is shown in Table 2. The most predominant acid was the lactic acid with S2 having the highest. There was however, significant difference ($p<0.05$) in the lactic, oxalic and citric acid contents of the beverage (Chelule et al., 2010; Mugula et al., 2003; Temitope & Taiyese). The production of organic acids are mostly due to utilization of carbohydrate (Chelule et al., 2010). However, there was no significant difference in the carbohydrate contents among the samples (S1 and S2, S3 and S4) (Table 1) which does not correspond to the total amount of acids produced (Table 2) can be ascribed to the choosiness in the nutritional requirements and the raw material utilization rate by the LAB (Li et al., 2014).

**Sensory analysis**

The preparation of most beverages are normally carried out by spontaneous fermentation with mixed cultures using yeasts or lactic acid-producing bacteria (Akpinar-Bayizit et al., 2010). In this regard, interactions among fermenters cannot be controlled during the fermentation process, which leads to variations in the product’s sensory characteristics, storage stability, composition, and fermentation profile (Sanni, 1993). Therefore, in this study pearl millet beverage was produced using individual *lactobacillus* species (*L. paracasei*, *L. brevis*, *L. platarum* and *L. fermentum*). The assessment of the fermented millet beverages color by the panelists showed that, there was no significant difference ($p<0.05$) among *L. brevis*, *L. platarum* and *L. fermentum* fermented beverages (Fig. 2). It was however noticed there was significant difference between *L. paracasei* and *L. platarum* fermented beverages.

**Proximate analysis**

The proximate compositions of the beverages are shown in Table 1. The proximate compositions of the fermented beverages (S1, S2, S3 and S4) were significant different ($p<0.05$) from the unfermented beverage (UF) with the exception of percentage moisture and fat in few instances (Table 1). This implies that fermentation brought about appreciable changes in the beverages (Saleh, Zhang, Chen, & Shen, 2013). The results (Table 1) showed a significantly ($p<0.05$) decrease in carbohydrate content in S1, S2, S3 and S4 than UF with a corresponding increase in total soluble sugars. This could be due to amylolytic action of the LAB during the fermenting (Arora, Jood, & Khetarpaul, 2011). Our findings are in line with those of Osman (2011), Sripriya, Antony, and Chandra (1997) and (Saleh et al., 2013). Like the carbohydrate contents, there was a reduction in the total reducing sugars in the fermented samples as a result of the simultaneous metabolism of the sugars by the LAB into organic acids (Akpinar-Bayizit, Yilmaz-Erzan, & Ozcan, 2010) and for their growth (Gänzlé, Vermeulen, & Vogel, 2007). Although S1 have a lowest TRS (1.30 %), its pH was slightly higher after the fermentation. This may be ascribed to the choosiness in the nutritional requirements and the raw material utilization rate by the LAB (Li et al., 2014).

**Table 1**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proximate Composition</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Fat</th>
</tr>
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<tbody>
<tr>
<td>UF</td>
<td></td>
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<td></td>
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<tr>
<td>S1</td>
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<td>S2</td>
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<td>S3</td>
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<tr>
<td>S4</td>
<td></td>
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</table>

**Fig. 1** Time course change of pearl millet beverage fermented with lactic acid bacteria for 48 h: (a) growth dynamics (b) pH of pearl millet beverage

S1 – Millet beverage fermented with *L. brevis*, S2 – Millet beverage fermented with *L. plantarum*, S3 - Millet beverage fermented with *L. paracasei*, S4 - Millet beverage fermented with *L. fermentum*
Results demonstrated that there were no significant differences among the lactic acid producing bacteria in the fermentation process. The results demonstrated that there were no significant (p<0.05) differences among L. brevis, L. paracasei, L. fermentum in terms of the taste of the beverage. However, L. paracasei was observed to significantly lower compared to the rest of the treatments with a score of 6.9. The assessment on the mouthfeel followed similar trend as that of the taste except that there was significant difference between L. paracasei and L. brevis. This finding revealed that the different lactobacillus strains used in this study did not significantly impact on the mouthfeel of the millet beverage even though S2 was higher than S1.

### Table 1 Proximate composition of fermented millet beverage

<table>
<thead>
<tr>
<th>Component (%)</th>
<th>UF</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>81.25 ± 0.35&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.30 ± 0.42&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;d&lt;/sup&gt;</td>
<td>82.10 ± 0.28&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.45 ± 0.21&lt;sup&gt;d&lt;/sup&gt;&lt;sup&gt;e&lt;/sup&gt;</td>
<td>83.25 ± 0.35&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>7.00 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.32 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.40 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.56 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.61 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>5.08 ± 0.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.60 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.05 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.45 ± 0.08&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>7.28 ± 0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>3.41 ± 0.02&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>3.44 ± 0.04&lt;sup&gt;ace&lt;/sup&gt;</td>
<td>3.37 ± 0.03&lt;sup&gt;bdf&lt;/sup&gt;</td>
<td>3.44 ± 0.03&lt;sup&gt;bde&lt;/sup&gt;</td>
<td>3.24 ± 0.04&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>0.28 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.33 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.40 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.35 ± 0.01&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>0.36 ± 0.01&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>TRS</td>
<td>2.64 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.30 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.94 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.66 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TSS</td>
<td>3.22 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.54 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.51 ± 0.03&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>3.39 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.56 ± 0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± standard deviation of replicates (n=3)

Parameters with same the superscripted alphabets in the same row implies no significant difference at p<0.05.

TRS – Total reducing sugar, TSS – Total soluble solid, UM – Unfermented millet beverage, S1 – Millet beverage fermented with L. brevis, S2 – Millet beverage fermented with L. paracasei, S3 – Millet beverage fermented with L. plantarum, S4 – Millet beverage fermented with L. fermentum

### Table 2 Organic acids of fermented millet beverage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oxalic acid (g/l)</th>
<th>Pyruvic acid (g/l)</th>
<th>Lactic acid (g/l)</th>
<th>Acetic acid (g/l)</th>
<th>Citric acid (g/l)</th>
<th>Succinic acid (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.24 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.11 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 ± 0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.17 ± 0.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2</td>
<td>0.32 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.81 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.32 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>S3</td>
<td>0.16 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.11 ± 0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.35 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.93 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11 ± 0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>S4</td>
<td>0.29 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.13 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.52 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.80 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.35 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 ± 0.00&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± standard deviation of replicates (n=3)

Parameters with same the superscripted alphabets imply no significant difference at p<0.05.

S1 – Millet beverage fermented with L. brevis, S2 – Millet beverage fermented with L. paracasei, S3 – Millet beverage fermented with L. plantarum, S4 – Millet beverage fermented with L. fermentum
followed by S3. This is because the strains used in this study were all amylase-producing LAB. This may explain the reason why the beverages fermented using the LAB were significantly different \((p<0.05)\) than the control. Besides, LABs contribute to the aroma and taste of fermented products \((\text{Leroy & De Vuyst, 2004})\). LABs acidify food, and gives it a tangy lactic acid taste, and produce aromatic compounds such as amino acids upon further bioconversion. This may also explain the reason why the panelists preferred the fermented beverages and rated both aroma and taste higher than the unfermented beverage (control). With respect to the overall acceptability of the millet beverages, it was revealed that the \(L.\ platatarum\) fermented millet beverage was the most preferred followed by \(L.\ brevis\). Furthermore, the results of the sensory characteristics, appeared the panelists preferred millet beverage with good color, taste and aroma. In totality, the findings indicated that differences in sensorial properties were significantly \((p<0.05)\) associated with the kind of lactic acid bacteria used in the fermentation of the beverage. Even though the ratings of the sensory parameters were not within wide limits some significant differences \((p<0.05)\) were noticed among the samples. This may be due to the fact that fermentation is important for improving product sensory attributes as well as safety, especially by reduction of toxic cyanogenic glucosides \((\text{Kostinek et al., 2005})\). The results obtained showed that the beverage has the potential of penetrating the local market of the area.

**Principal component analysis**

Principal component analysis (PCA) was carried out to establish the key features of each sample using the proximate composition \((\text{Table 1})\), the organic acid content \((\text{Table 2})\) and the sensory attributes \((\text{Fig. 2})\) of the samples. The first two principal components \((\text{PC1 and PC2})\) were able to explain 81.47% of the total variance. PC1 accounted for 44.03% and noticeably categorized the beverages into two \((\text{S2, S4})\) and \((\text{S1, S3})\) \((\text{Fig. 3a})\). PC2, which accounted for 37.44% of the variance, distinctly characterized S1 and S2 from S3 and S4 \((\text{Fig. 3a})\). From the loading values \((\text{Table 3})\) of each attributes the samples were split into four groups \((\text{Fig. 3a})\) and their characteristics as highlighted in \(\text{Fig. 3b}\). The first group was on the positive side of PC1 and negative side of PC2 was S4 which was characterized by their pyruvic acid, protein, carbohydrate, total reducing sugars and mouthfeel. The second group S3 characterized by its fat content was located on the negative sides of PC1 and PC2. The third group was situated on the negative side of PC1 and positive side of PC2 was the S1 was characterized by its organic acids \((\text{succinic, citric and acetic})\) \((\text{Fig. 3b})\). The last group situated on the positive side of PC1 and PC2 was the S2. This beverage was characterized by lactic acid, oxalic acid, TSS, moisture, color, ash, aroma, taste and acceptability. Though S4 was characterized by mouthfeel \((\text{Fig. 3})\), its mouthfeel score \((\text{Fig. 2})\) was lower than that S3 \((\text{Fig. 2})\). This variation may be due to the subjective nature of sensory analysis using human beings.

**Conclusion**

In the current study, we fermented millet using four strains of LAB. The study evaluated the kinetics of the growth of the LAB and pH during the fermentation. It also assessed the proximate composition, the organic acids composition, sensory attributes and categorized the samples. Our results showed that LAB performed during the fermentation and were able to improve on the nutritional content of the beverages. The production of organic acids enhanced the sensory acceptability. Comparing the
performance of the LAB and the other parameters assessed, we conclude that *L. plantarum* was the most suitable strain in the fermentation of the millet beverage and might have the ability for a possible industrial application in the production of lactic acid fermented millet beverage. Based on our study it can be concluded that fermentation using single strains is a promising technique that can be used to prepare fermented beverage with high nutritional and sensory characteristic from millet grains. However, further work on optimizing the fermentation conditions and *in vitro* as well as *in vivo* assessment on the functionality of the lactic-acid-fermented millet beverage is highly recommended.

**Fig. 3** Principal component analysis of millet beverage showing a) Score scatter plot and b) loading plot

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