(Research Article)

Evaluation of potential lactic acid bacteria single starter cultures for production of traditional Ivorian cereal beverages

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Abstract

Twenty-three lactic acid bacteria (LAB) previously isolated from tchapalo process were evaluated as single starter cultures for traditional Ivorian beverages production. These LAB were inoculated separately into sorghum wort and fermented at 30 °C for 12 h. During fermentations, pH, titratable acidity, and LAB growth were determined. After that, the six better bacteria had been used to produce controlled sour wort, sweet wort and tchapalo. Then, pH, titratable acidity, total soluble solids, and organic acids were analyzed. Their antimicrobial activities also were tested. Within 6 h of fermentation, out of twenty-three bacteria, only six (26.08%) of strains namely L. fermentum strains S6, S42 and S45, P. acidilactici strains S7 and S52 and P. pentosaceus strain S5 accelerated fermentation by dropping of ΔpH to ≥ 1.5 units and increasing of ΔAT to ≥ 0.15%. Lactobacillus fermentum strain S6 carried out his exponential growth rate from 0.18 log CFU/mL to 2.67 log CFU/mL during 2 to 6 h. The sour wort obtained by using of these strains were like those of controls. Tartaric and lactic acids were detected in all the fermented beverages with single starter cultures with a predominance of lactic acid, but their contents varied according to starter cultures. Some sweet wort and tchapalo produced with single starter cultures were able to inhibit the growth of Escherichia coli, Salmonella typhi and Staphylococcus aureus. These six LAB would be used single to improve safety of sweet wort and tchapalo.

Keywords: Lactic acid bacteria; Starter cultures; Fermentation; Sorghum wort; Sweet wort; Tchapalo

1. Introduction

Sorghum is an important cereal in Africa regions. Most of this staple, produced in Côte d’Ivoire or imported from neighboring countries, is used to produce tchapalo, a traditional cloudy beer containing suspended solids and yeast [1, 2]. This traditional beverage is obtained from alcoholic fermentation of sweet wort. The sweet wort, which is a non-alcoholic beverage, is obtained by spontaneous lactic fermentation and/or back-slopping of the sweet wort [3, 4]. Sweet wort is generally consumed by women, infants, children, and non-alcoholic consumers. Both beverages have nutritional values helping to improve the diet of consuming populations. In addition, therapeutic properties are attributed to them because of their laxative, antimalarial and anti-hemorrhoidal properties [4-6].

The production of tchapalo and sweet wort originate from Northern Côte d’Ivoire and are mainly produced by women. These women have now made it a real economic activity generating income in all areas of the country and particularly in Abidjan, the economic capital [3, 5, 6]. Unfortunately, tchapalo and sweet wort are often produced under poor hygienic conditions with rudimentary equipment, tedious and costly operations, and the use of unselected dried traditional yeasts from previous alcoholic fermentations. The spontaneous fermentation also depends of environmental
microorganisms containing benefic and pathogenic microorganisms. These fermentations do not always guarantee product quality and safety and thus necessitates the use of pure starter cultures.

According to Holzapfel [7], the use of starter cultures containing microorganisms isolated from fermented products for fermentation would be an appropriate approach for the control and optimization of the fermentation process in order to alleviate the problems of variations in organoleptic quality and microbiological stability observed in African indigenous fermented foods. Previous studies have shown that lactic acid bacteria (LAB) are involved in the traditional Ivoirian sorghum-based beverages process, mainly during the spontaneous lactic fermentation [3]. The objective of the present study was to assess the selected LAB isolated from fermented sorghum-based beverages in Côte d’Ivoire for their performance as single strain starter cultures for production of sweet wort and tchapalo. We also determined whether the beverages obtained from the different starters inhibit some foodborne pathogens.

2. Material and methods

2.1. Samples collection

Red sorghum grains and samples of sour wort, sweet wort and tchapalo of three processes produced by a traditional brewer at Abobo Pk18 (Abidjan, Southern Côte d’Ivoire) using the red sorghum were collected. These samples were used as controls for samples that were prepared in the laboratory. They were collected in sterile bottles, labelled, and then transported to the laboratory in an icebox containing a freeze pack.

2.2. Strains and growth conditions

Twenty-three LAB (thirteen Lactobacillus fermentum, six Pediococcus acidilactici, three Pediococcus pentosaceus and one Lactobacillus plantarum) isolated from tchapalo process in previous study [8] were used to evaluate as single starter cultures for the different control fermentations. These strains were selected because they inhibited some foodborne pathogens by organic acids production. LAB strains were grown on MRS agar at 30 °C for 48 h. An isolated colony was picked up and sub-cultured in MRS broth at 30 °C for overnight. After growth, each LAB culture was centrifuged at 4000 × g for 10 min and the pellet was collected for wort inoculation. Staphylococcus aureus, Escherichia coli and Salmonella typhi were used as foodborne pathogens. They were sub-cultured at 37 °C in nutrient broth, after growing on nutrient agar. All these strains were obtained into the Laboratory of Food Biotechnology and Microbiology, faculty of food sciences and technologies, Nangui Abrogoua University, Côte d’Ivoire.

2.3. Laboratory preparation of beverages

2.3.1. Preparation of wort

Beverages were prepared according to brewers tchapalo process described by Aka et al. [1] using pure single starter cultures (Fig. 1). Red sorghum (Sorghum bicolor (L.) Moench) grains (5 kg) collected from traditional brewer were sorted manually to remove debris. Then, the grains were steeped in water (20 L) at 37 °C for 10 h. After steeping, they were drained and were germinated at 37 °C for 3 days before drying at ambient temperature for 24 h. The dried malt obtained was milled to give malted sorghum flour. Then, 3.4 kg of malted sorghum flour were mixed with 20 L water containing 1% of grinding the bark of Anogeissus leocarpus and then left in decantation during 45 min. Subsequently, 10 L of the supernatant were removed while the sediment was boiled at 100 °C for 1 h 30 min to gelatinize malt starch. Then boiled sediment was mixed with the supernatant to give the wort. Finally, this wort was distributed in flasks (500 mL per flask) and was pasteurized at 63 °C for 30 min.

2.3.2. Inoculation and fermentation assays

The wort was inoculated with 1 × 10⁶ CFU/mL of each single LAB starter culture and incubated at 30 °C for 12 h. For initial incubation and every 2 h, 15 mL was sampled under aseptic conditions for physico-chemical and microbiological analysis. After that, sour wort was obtained. Three trial fermentations were carried out.

2.3.3. Sweet wort obtention and alcoholic fermentation

The sour wort was boiled to obtain sweet wort whose total soluble solids (TSS) content was comprised between 11 and 14 °Brix and then cooled. Finally, 1% commercial Saccharomyces cerevisiae starter was inoculated to sweet wort for alcoholic fermentation during 12 h according to N’Guessan et al. [9]. The product obtained after alcoholic fermentation was tchapalo. Three trial fermentations were carried out.
Figure 1 Diagram of tchapalo process in the laboratory using single starter cultures

2.4. Physico-chemical analysis

pH was measured using a digital pH-meter (P107 Consort). Acidification rate was calculated as ΔpH according to Ayad et al. [10] (ΔpH = pH at time – pH zero time). The Titratable Acidity (TA), expressed as a percentage of lactic acid, was determined by titration of 5 mL sample against 0.1 N NaOH using phenolphthalein as indicator. Acid production rate was calculated as ΔAT (ΔAT = AT at time – AT zero time). The Total Soluble Solids (TSS) content, expressed as °Brix value, was determined in each sample using a hand refractometer (ATAGO, N-20E, Japan). Two independent measurements were made for each sample. Organic acids were determined by High-Performance Liquid Chromatograph system (HPLC, LC-6A, Shimadzu corporation, Japan) according to Aka et al. [1]. Analyses were carried out with an ion- exclusion ORH-801 column (300 mm × 6.5 mm) (Interchrom, France) preceded by a Universal Guard
Cartridge-Holder column. The HPLC was equipped of a Shimadzu LC-6A pump. Column effluents were monitored by an UV detector (SPD-6A, Shimadzu Corporation, Japan) set at 210 nm. The mobile phase (0.004N H2SO4) used at a flow rate of 0.8 mL/min was filtered through a 0.45 μm Millipore membrane filter (Sartorius AG, Goettingen, Germany). Each sample was injected in duplicate. Organic acids were identified by comparing their retention times with those of standards.

2.5. Microbiological analysis

For enumeration of LAB, 10 mL of sample was homogenized in 90 mL sterile peptone water (pH 7.0) to obtain a 1:10 dilution. Further 10-fold dilutions were prepared from this and 1 mL of an appropriate dilution was spread onto MRS agar. Plates were incubated at 30 °C for 48 h and colony forming units (CFU/mL) estimated. The population growth dynamics (ΔGrowth) of the LAB was calculated as ΔGrowth (ΔGrowth = Growth at time – Growth zero time). Antibacterial activity was assayed using an agar diffusion method described by Arici et al. [11]. Ten (10) mL of sour wort, sweet wort and tchapalo resulting from inoculation of each LAB strain were centrifuged (4000 × g, TGL-16 M) at 4 °C for 10 min. The supernatants were filtered through a 0.20 μm pore size filter (Corning syringe filters, Sigma-Aldrich, Germany). An overnight culture of the target strain was diluted in sterile Mueller-Hinton Medium and 200 µL of approximately 1 × 10⁶ CFU/mL were spread on solid Mueller-Hinton Medium. Filtered samples (100 μL) were spotted in wells (5 mm in diameter) on the agar plate. The plates were placed at 4 °C for 1 h and then incubated at 37 °C for 24 h. The appearance of the clear zone around the wells of the growth of target strain was used to indicate inhibitory activity. Therefore, the diameters (mm) of these zones were measured and recorded. Antibacterial tests were carried out in twice.

2.6. Statistical analysis

Results were statistically evaluated by one way analysis of variance (ANOVA) and Duncan’s multiple range test with the software Statistica version 7.1. Differences were considered significant at p < 0.05 level.

3. Results

3.1. Selection of starter cultures

3.1.1. Changes in pH and titratable acidity during sorghum wort fermentations using single starter cultures as inoculum

Changes in pH and titratable acidity during sorghum wort fermentations using single starter cultures as inoculum were shown in Fig. 2 and Fig. 3 respectively. The pH of sorghum wort dropped whereas acidity increased. However, the acid production varied within strains of the same species and between species also. The ΔpH of wort from strains varied significantly (p < 0.05) from 0 unit to 2.15 units within 12 h of fermentation. Twenty-three bacteria, only six (26.08%) of strains namely L. fermentum strains S6, S42 and S45, P. acidilactici strains S7 and S52 and P. pentosaceus strain S5 accelerated fermentation by dropping of ΔpH to ≥ 1.5 units and increasing of ΔAT to ≥ 0.15%. After 8 h of fermentation, L. fermentum strains S6, S8, S9, S11, S13, S41, S42 and S45 dropped the pH to ≥ 1.7 units while increasing the ΔAT to ≥ 0.17%. In the same time, P. acidilactici strains, P. pentosaceus strain and L. plantarum accelerated less fermentation of the sorghum wort than L. fermentum strains. A decrease of ΔpH to ≤ 1.7 units was observed except P. acidilactici strain S7 and P. pentosaceus strain S5 which were dropped pH to 1.74 and 1.96 units respectively. In short, ten strains out of twenty-three (43.47%) quickly dropped the pH within 8 h of fermentation. After 12 h of fermentation, only L. fermentum strains S6, S42 and S45 attained a ΔpH to ≥ 2.1 units and the increasing of ΔAT to ≥ 0.3%. However, P. acidilactici strain S5 decreased ΔpH to 2.03 units and increased ΔAT to 0.33%. Pediococcus acidilactici strain S5 increased ΔAT to 0.35%; while, the decrease of ΔpH of P. pentosaceus strains S5 and S47 were to 2.11 and 1.99 units respectively with an increase of ΔTA to 0.39 and 0.26 % respectively. Furthermore, L. plantarum attained a ΔpH to 1.9 units and ΔAT to 0.24%.
Figure 2 Changes in pH during sorghum wort fermentations using single starter cultures as inoculum. A = *Lactobacillus fermentum* strains; B = *Pediococcus acidilactici* strains; C = *Pediococcus pentosaceus* and *Lactobacillus plantarum* strains.
Figure 3 Changes in titratable acidity during sorghum wort fermentations using single starter cultures as inoculum. A = *Lactobacillus fermentum* strains; B = *Pediococcus acidilactici* strains; C = *Pediococcus pentosaceus* and *Lactobacillus plantarum* strains.
3.1.2. Growth dynamic of LAB species during fermentation

Population growth dynamics of the LAB used as single starter cultures in the sorghum wort fermentations were monitored from 0 h to 12 h (Fig. 3). Results indicated that, generally, a lag phase was observed until to 2 h or 4 h according to LAB followed by an exponential growth. Then, came the stationary phase which ends with the decrease phase. Most of LAB achieved their exponential growth between 8 h and 10 h increasing their growth rate from 2.14–3.65 log CFU/mL to 2.9–3.99 log CFU/mL. Thereby, during exponential phase, the growth dynamic of *L. fermentum* group 1 increased from 0.05–0.18 log CFU/mL to 2.21–3.07 log CFU/mL during 2 h to 8 h (Fig. 3A1). However, *L. fermentum* strain S_6_ carried out his growth rate from 0.18 log CFU/mL to 2.67 log CFU/mL during 2 h to 6 h. During 8 h to 10 h, the growth rate of *L. fermentum* group 1 increased slightly from 2.14–3.07 log CFU/mL to 2.14–3.42 log CFU/mL; but *L. fermentum* strain S_46_ remained constant from 8 h to 12 h of fermentation with a growth rate of 2.14 log CFU/mL. A decrease of the growth rate was observed from 2.14–3.42 log CFU/mL to 1.78–2.55 log CFU/mL between 10 h to 12 h of fermentation except *L. fermentum* strains S_118_ and S_41_ which the growth rate increased slightly until to 3.1 and 3.59 log CFU/mL respectively. On the other hand, 2 h to 8 h growth rate of *L. fermentum* group 2 were lower than those of *L. fermentum* group 1 (Fig. 3A2). Until 12 h of fermentation, the stationary phase was not observed. At the level of sorghum wort that was inoculated with *P. acidilactici* strains, the exponential phase was taken place from 2 h to 8 h (Fig. 3B). During this phase, the growth rate went from 0.03–0.19 log CFU/mL to 1.85–3.65 log CFU/mL with the largest values observed in fermented wort from *P. acidilactici* strains S_52_ and S_14_ (3.39 and 3.65 log CFU/mL respectively). Moreover, *L. plantarum* alone had a growth dynamic higher than all species which achieved during 6 h time of fermentation with a value of 3.88 log CFU/mL (Fig. 3C). On the other hand, the highest growth rate for *P. pentosaceus* strain S_5_ was observed at 8 h of fermentation (3.17 log CFU/mL, Fig. 3C).

![Growth rate of LAB species during sorghum wort fermentations using single starter cultures. A1 = Lactobacillus fermentum strains group 1; A2 = Lactobacillus fermentum strains group 2; B = Pediococcus acidilactici strains; C = Pediococcus pentosaceus and Lactobacillus plantarum strains.](image)
3.2. Characteristics of beverages from controlled fermentations

3.2.1. Biochemical characteristics

The biochemical characteristics of beverages obtained by sorghum wort fermentations using L. fermentum strains S6, S42 and S45, P. acidilactici strains S7, S52 and P. pentosaceus strain S5 as single starter cultures were shown in Tables 1, 2 and 3. All pH of sour wort from fermentations with L. fermentum strains S6, S42 and S45, P. acidilactici strains S7, S52 and P. pentosaceus strain S5 used as inoculum were lower with values between 3.6 and 4.0; while the pH of sour wort from brewer and spontaneous fermentation carried out in the laboratory used as controls were the same (pH 3.7±0.6 and 3.7±0.8 respectively). However, there was not significant difference (P > 0.05). On the other hand, the sour wort from L. fermentum strains S6 and S45 had the highest titratable acidity (0.4±0.1%, Table 1). The sweet wort from sour wort fermented with single LAB strains were also acid (pH: 3.3±0.3–3.9±0.1 and titratable acidity: 0.3±0.1%, Table 2). The titratable acidity of sweet wort from brewer and laboratory were comparative to those of starters; they were not significantly different (p > 0.05). Only tchapalo from spontaneous fermented wort carried out in the laboratory had the highest pH (4.5±0.5). This value was significantly different (p < 0.05) to others. The lowest pH observed was those of the tchapalo obtained by brewer spontaneous fermented wort (pH 3.7±0.4). But this value was comparative to those of starters (Table 3).

The content of soluble solids of the sour wort from sorghum wort fermentations using single starter cultures varied between 5.0±0.0 °Brix and 5.5±0.7 °Brix, but there was not significant difference (P > 0.05) between these values (Table 1). Those of brewer spontaneous fermented sour wort was 6.0±1.4 °Brix. However, the increase of content of soluble solids was observed in the sweet wort obtained according to starter cultures. The highest content of soluble solids was noted in sweet wort fermented with P. acidilactici strains S7 (13.0±1.4 °Brix, Table 2); while the lowest content of soluble solids was observed in sweet wort fermented with P. acidilactici strain S52 (10.5±0.7 °Brix). All the content of soluble solids of tchapalo were lower than those of sweet wort. On the other hand, the content of soluble solids of tchapalo from L. fermentum S5 (4.5±0.7 °Brix), P. acidilactici S7 (5.0±0.0 °Brix), L. fermentum S50 (4.3±0.4 °Brix) and P. acidilactici S52 (4.3±1.8 °Brix) were significantly comparable with tchapalo from brewer spontaneous fermented (5.5±0.7 °Brix).

Tartaric and lactic acids were detected in all the fermented sour wort with single starter cultures with a predominance of lactic acid, but their contents varied according to starter cultures (Table 1). Thereby, the highest contents of tartaric acid were found in sour wort from P. pentosaceus S5 (4.01±0.52 g/L), P. acidilactici S7 (3.81±0.03 g/L) and L. fermentum S52 (3.68±0.32 g/L). These values were significantly different (P < 0.05) to the brewer spontaneous fermented sour wort (0.67±0.24 g/L). Otherwise, sour wort produced with L. fermentum S5 (9.74±0.98 g/L), L. fermentum S52 (9.46±0.23 g/L) and P. pentosaceus S5 (8.12±0.07 g/L) contained higher lactic acid than those of other sour wort but there was not significantly different (P > 0.05). Acetic acid was only detected in fermented sour wort with L. fermentum strains (0.06±0.01 - 0.07±0.01 mg/L) and sour wort from spontaneous fermentation carried out in the laboratory (0.17±0.03 mg/L). On the other hand, fumaric acid was not detected in sour wort produced with P. acidilactici strains. In all sour wort, contents of organic acids were strongly high than in sour wort (Table 2). Lactic acid was higher than other organic acids. The lactic acid content of sour wort from P. acidilactici strain S7 (5.17±0.02 g/L) was highest and significantly different (P < 0.05) of those of brewer spontaneous fermented sour wort (19.78±0.93 g/L) and the all others sour wort. In the tchapalo, no acetic acid was detected (Table 3). On the other hand, there were tartaric, lactic and fumaric acids whose content is lower than those of sour wort from which they were derived. Thus, the lowest lactic acid content was observed in the tchapalo from L. fermentum strains S45 (9.77±0.23 g/L). However, this content did not vary significantly (P > 0.05) in tchapalo obtained by spontaneous fermentation from the brewer (11.61±0.28 g/L).
Table 1 Biochemical characteristics of sour wort from sorghum wort fermentations using single starter cultures

| Characteristics | Sour wort | Controls |
|-----------------|-----------|----------|
|                 | Single starter cultures | | |
|                 | S<sub>5</sub> | S<sub>6</sub> | S<sub>7</sub> | S<sub>42</sub> | S<sub>45</sub> | S<sub>52</sub> | T<sub>F</sub> | T<sub>L</sub> | TB |
| pH              | 3.6±0.3<sup>a</sup> | 3.9±0.3<sup>a</sup> | 3.5±0.1<sup>a</sup> | 3.8±0.4<sup>a</sup> | 4.0±0.8<sup>a</sup> | 3.7±0.1<sup>a</sup> | 3.7±0.6<sup>a</sup> | 3.7±0.8<sup>a</sup> | 6.6±0.0<sup>c</sup> |
| AT (% lactic acid) | 0.3±0.1<sup>a</sup> | 0.4±0.1<sup>a</sup> | 0.2±0.0<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.4±0.1<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.2±0.1<sup>a</sup> | 0.2±0.1<sup>a</sup> | 0.1±0.0 |
| TSS (°Brix)     | 5.0±0.0<sup>a</sup> | 5.0±0.0<sup>a</sup> | 5.5±0.7<sup>a</sup> | 5.0±0.0<sup>a</sup> | 5.0±0.0<sup>a</sup> | 5.5±0.7<sup>a</sup> | 6.0±1.4<sup>a</sup> | 5.0±0.0<sup>a</sup> | 5.0±0.0<sup>a</sup> |
| Tartaric acid (g/L) | 4.01±0.52<sup>c</sup> | 1.44±0.06<sup>b</sup> | 3.81±0.03<sup>c</sup> | 3.68±0.32<sup>c</sup> | 1.36±0.4<sup>b</sup> | 2.36±0.23<sup>b</sup> | 0.67±0.24<sup>a</sup> | 2.29±0.17<sup>b</sup> | 1.92±0.44<sup>b</sup> |
| Acetic acid (mg/L) | 0.16±0.02<sup>b</sup> | 0.49±0.04<sup>b</sup> | 0 | 0.37±0.05<sup>a</sup> | 4.73±0.34<sup>c</sup> | 4.4±1.1<sup>b</sup> |

Values are expressed as means ± standard deviation for three independent fermentations. Means values with same letter in a line for each parameter are not significantly different (P > 0.05). AT: titratable acidity, TSS: total soluble solids; S<sub>5</sub>, S<sub>6</sub>, S<sub>7</sub>: L. fermentum strains, S<sub>42</sub>, S<sub>45</sub>: P. acidilactici strains, S<sub>52</sub>: P. pentosaceus strain

Table 2 Biochemical characteristics of sweet wort from sorghum wort fermentations using single starter cultures

| Characteristics | Sweet wort | Controls |
|-----------------|-----------|----------|
|                 | Single starter cultures | | |
|                 | S<sub>5</sub> | S<sub>6</sub> | S<sub>7</sub> | S<sub>42</sub> | S<sub>45</sub> | S<sub>52</sub> | T<sub>F</sub> | T<sub>L</sub> | TB |
| pH              | 3.3±0.3<sup>a</sup> | 3.9±0.3<sup>a</sup> | 3.8±0.2<sup>a</sup> | 3.8±0.1<sup>a</sup> | 3.9±0.1<sup>a</sup> | 3.4±0.4<sup>a</sup> | 3.5±0.1<sup>a</sup> | 3.9±1.2<sup>a</sup> | 6.6±0.0<sup>c</sup> |
| AT (% lactic acid) | 0.3±0.1<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.3±0.2<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.1±0.0 |
| TSS (°Brix)     | 11.5±2.1<sup>b</sup> | 12.3±1.8<sup>b</sup> | 13.0±1.4<sup>b</sup> | 11.1±1.3<sup>b</sup> | 11.8±3.2<sup>b</sup> | 10.5±0.7<sup>b</sup> | 12.2±1.7<sup>b</sup> | 14.0±2.1<sup>b</sup> | 5.0±0.0<sup>a</sup> |
| Tartaric acid (g/L) | 6.88±0.22<sup>c</sup> | 5.58±0.47<sup>c</sup> | 12.13±2.12<sup>d</sup> | 12.07±0.44<sup>d</sup> | 6.97±1.1<sup>c</sup> | 6.28±1.32<sup>c</sup> | 2.38±0.37<sup>b</sup> | 6.97±1.64<sup>c</sup> | 1.92±0.44<sup>a</sup> |
| Acetic acid (mg/L) | 0.15±0.05<sup>a</sup> | 0.16±0.02<sup>a</sup> | 0.17±0.02<sup>a</sup> | 0 | 0 | 1.1±0.03<sup>b</sup> | 0 |
| Lactic acid (g/L) | 35.93±0.23<sup>f</sup> | 18.59±0.1<sup>c</sup> | 51.73±0.02<sup>f</sup> | 23.16±0.33<sup>d</sup> | 9.79±0.03<sup>b</sup> | 30.69±0.01<sup>e</sup> | 19.78±0.93<sup>d</sup> | 9.69±0.01<sup>b</sup> | 0.86±0.01<sup>a</sup> |
| Fumaric acid (mg/L) | 6.42±0.18<sup>c</sup> | 7.17±0.1<sup>d</sup> | 0.07±0.02<sup>a</sup> | 8.72±1.08<sup>b</sup> | 4.73±0.2<sup>b</sup> | 0.77±0.11<sup>a</sup> | 4.94±0.32<sup>b</sup> | 6.48±0.4<sup>c</sup> | 4.4±1.1<sup>b</sup> |

Values are expressed as means ± standard deviation for three independent fermentations. Means values with same letter in a line for each parameter are not significantly different (P > 0.05). AT: titratable acidity, TSS: total soluble solids; S<sub>5</sub>, S<sub>6</sub>, S<sub>7</sub>: L. fermentum strains, S<sub>42</sub>, S<sub>45</sub>: P. acidilactici strains, S<sub>52</sub>: P. pentosaceus strain
### Table 3 Biochemical characteristics of tchapalo from sorghum wort fermentations using single starter cultures

| Characteristics | Tchapalo |
|-----------------|----------|
|                 | Single starter cultures | Controls |
|                 | S5 | S6 | S7 | S42 | S45 | S52 | TF | TL | TB |
| pH              | 3.9±0.3<sup>ab</sup> | 4.0±0.1<sup>ab</sup> | 3.8±0.3<sup>ab</sup> | 3.9±0.1<sup>ab</sup> | 3.9±0.1<sup>ab</sup> | 3.8±0.0<sup>ab</sup> | 3.7±0.4<sup>a</sup> | 4.5±0.5<sup>b</sup> | 6.6±0.0<sup>c</sup> |
| AT (% lactic acid) | 0.4±0.1<sup>a</sup> | 0.4±0.1<sup>a</sup> | 0.4±0.1<sup>a</sup> | 0.4±0.0<sup>a</sup> | 0.4±0.1<sup>a</sup> | 0.4±0.1<sup>a</sup> | 0.4±0.1<sup>a</sup> | 0.3±0.1<sup>a</sup> | 0.1±0.0 |
| TSS (°Brix)     | 3.3±0.4<sup>a</sup> | 4.5±0.7<sup>ab</sup> | 5.0±0.0<sup>ab</sup> | 3.5±0.7<sup>a</sup> | 4.3±0.4<sup>ab</sup> | 4.3±1.8<sup>ab</sup> | 5.5±0.7<sup>b</sup> | 6.0±0.0<sup>b</sup> | 5.0±0.0<sup>ab</sup> |
| Tartaric acid (g/L) | 3.97±1.08<sup>b</sup> | 4.91±0.8<sup>b</sup> | 13.29±0.23<sup>d</sup> | 9.01±0.84<sup>c</sup> | 3.76±0.41<sup>b</sup> | 4.78±0.08<sup>b</sup> | 0 | 3.56±0.49<sup>b</sup> | 1.92±0.44<sup>a</sup> |
| Acetic acid (mg/L) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,00 | 0 |
| Lactic acid (g/L) | 16.94±0.07<sup>de</sup> | 14.27±0.14<sup>d</sup> | 17.24±1.22<sup>e</sup> | 11.83±3.32<sup>c</sup> | 9.77±0.23<sup>b</sup> | 13.79±0.96<sup>cd</sup> | 11.61±0.28<sup>bc</sup> | 9.67±0.25<sup>b</sup> | 0.86±0.01<sup>a</sup> |
| Fumaric acid (mg/L) | 11.97±0.67<sup>c</sup> | 0 | 0 | 4.76±0.47<sup>h</sup> | 6.29±1.02<sup>c</sup> | 0.67±0.34<sup>a</sup> | 8.25±0.94<sup>d</sup> | 6.29±1.2<sup>c</sup> | 4.4±1.1<sup>b</sup> |

Values are expressed as means ± standard deviation for three independent fermentations. Means values with same letter in a line for each parameter are not significantly different (P > 0.05). AT: titratable acidity, TSS: total soluble solids; S6, S42, S45: L. fermentum strains, S7, S52: P. acidilactici strains, S5: P. pentosaceus strain.
3.2.2. Antibacterial properties

Inhibition spectra of sour wort, sweet wort and tchapalo produced with single starter cultures are shown in Table 4. Sour wort did not display bactericidal activity against the indicator strains. On the other hand, all sweet wort and tchapalo produced with single starter cultures were able to inhibit the growth of E. coli, S. typhi and S. aureus. The inhibition diameters were ranged from 11±1 to 23±6 mm according to sweet wort and indicator strains. The highest diameters were observed against E. coli (19±1 - 23±6 mm). All tchapalo exhibited also antibacterial activity against S. aureus (11±1 - 15±1 mm). However, only tchapalo from P. acidilactici S7, L. fermentum S45 and P. acidilactici S52 had antagonist activity against E. coli (14±1 - 15±1 mm). Salmonella typhi was the most sensitive indicator strain to tchapalo with single starter cultures.

Table 4 Antimicrobial activity of beverages from controlled fermentations against indicator strains

| Beverages | Indicator strains | Single starter cultures | Controls |
|-----------|------------------|-------------------------|----------|
|           |                  | S5  | S6  | S7  | S42 | S45 | S52 | TF  | TL  | TB  |
| Sour wort | E. coli          | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
|           | S. typhi         | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
|           | S. aureus        | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Sweet wort| E. coli          | 23±6| 22±6| 21±4| 23±6| 19±1| 23±6| 19±8| 21±4| 0   |
|           | S. typhi         | 15±1| 15±1| 17±2| 18±4| 19±5| 16±1| 15±2| 15±7| 0   |
|           | S. aureus        | 16±4| 12±0| 20±3| 17±1| 17±2| 18±4| 19±2| 11±1| 0   |
| Tchapalo  | E. coli          | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
|           | S. typhi         | 0   | 0   | 0   | 0   | 0   | 0   | 16±1| 0   | 0   |
|           | S. aureus        | 13±1| 13±1| 15±1| 12±1| 11±1| 13±1| 12±3| 11±1| 0   |

S5, S45, S42: L. fermentum strains, S7, S52: P. acidilactici strains, S6: P. pentosaceus strain

4. Discussion

The selection of LAB as starter cultures for cereal fermentation is a complex process, involving the evaluation of some desired metabolic traits and technological performances such as rapid acidification, rapid growth, production of antimicrobial compounds, reduction of hygienic risks [7, 12, 13]. When the lactic acid bacteria were used as single starter cultures in the sorghum wort for fermentations, pH of this dropped significantly at the end of fermentation whereas the titratable acidity increased. This can be explained by the fact that these bacteria used sugar, the main compound of sorghum wort, to produce organic acids which decreased the pH as described by many authors [15–16]. However, acid production varied within strains of the same species and between species also. Thereby, within 6 h of fermentation, out of twenty three bacteria, only six (26.08%) namely L. fermentum strains S6, S42 and S45, P. acidilactici strains S7 and S52 and P. pentosaceus strain S5 accelerated fermentation by dropping of ΔpH to ≥ 1.5 pH units and increasing of ΔAT to ≥ 0.15%. These bacteria are distinguished from others by quickly acidification. These results were comparable to those of Sawadogo-Lingani et al. [17]. During their study on technological properties of Lactobacillus fermentum isolates involved in spontaneous fermentation of dolo and pito wort to select starter cultures, authors have shown that the faster acidifying group of isolates (which represent 43.48%) had a acidification rate evaluated as ΔpH of 1.14 ± 0.15 pH unit after 6 h of fermentation. Other authors mentioned the highest acidification power of LAB in many fermented products [18–20].

Acidification was correlated with growth rate of LAB. The same observations were made when Viera-Dalodé et al. [21] have used LAB individually or in combination with yeasts as inoculum enrichment for controlled fermentation of gowé. Most of LAB achieved their exponential phase between 8 h and 10 h increasing their growth dynamic from 2.14–3.65 log CFU/mL to 2.9–3.99 log CFU/mL. On the other hand, after 6 h of fermentation, L. plantarum S15 achieved its exponential phase, but did not quickly acidify the sorghum wort unlike L. fermentum S6 which achieved its exponential phase at the same time and quickly acidified the sorghum wort. These results are contrary to those of Apaliya et al. [14], the microbial population in the fermentation of millet beverage with L. plantarum S2 was significantly higher than all the other treatments and his pH was the lowest also.
All the beverages i.e. sour wort, sweet wort and tchapalo from single starters LAB had an acid pH like those of brewer obtained by spontaneous fermentation [1, 4, 5, 22]. This acidity was desirable and wished. Indeed, Aka et al. [1] mentioned that in the tchapalo process, acidity of sour wort was obligatory and important because it determined the further process, organoleptic properties and the preserving of sweet wort and tchapalo. The content of soluble solids of the sweet wort were higher than those of sour wort because sour wort was cooked during 4 to 6 hours to sweet wort. This cooking leads to a concentration of soluble solids into the sweet wort [1]. Our finding was similar to those of Maoura et al. [23] which found that the soluble sugar had increased a lot after cooking initial wort to give cooked wort. All soluble solid contents of tchapalo were lower than those of sweet wort. This decrease was the result of the utilization of soluble solids as a carbon source by yeast to produce ethanol [2, 4, 23, 24]. The content of soluble solids of tchapalo from L. fermentum S6, L. fermentum S45, P. acidilactici S7 and P. acidilactici S52 were significantly comparable with tchapalo from brewer spontaneous fermented. All the fermented sour worts with single starter cultures contained organic acids specifically lactic acid reducing the pH of the fermenting wort. These organic acids gave the souring taste to sour wort which is characteristic of this beverage [25]. The lactic acid content of sweet wort was highest to those of sour wort. This highest content would develop the desired flavor and reduce pH thus preventing growth of undesirable microorganisms and contributing to the development of the desired sensory qualities [26]. In the tchapalo, there was not acetic acid detected. Our results were in agreement with those of Dje et al. [24], which worked on biochemical changes during alcoholic fermentation in the production of tchapalo. They found that organic acid (oxalic, citric, tartaric, malic, lactic, fumaric and propionic acids) were found in the wort and in tchapalo and increased or decreased during fermentation differently from one site to another. But acetic acid was not always detected in both the wort and the beer.

The beverages such as sweet wort and tchapalo produced with single starter cultures had the ability to inhibit E. coli, S. typhi and S. aureus. Their inhibitory effects could be related to their organic acids content and composition [1, 8, 26]. Indeed, Byakika et al. [26] reported that organic acids inhibit pathogens by entering into cells in an undissociated form and dissociating within the cytoplasm. This lowers the intracellular pH, and to maintain balance, cells use ATP to expel the excess hydrogen ions. This exhausts the cell energy required for growth and other metabolic processes resulting in death. Thus, this beverage property improved his safety. Other researchers showed antimicrobial activities of traditional food and beverages against both Gram-negative and Gram-positive bacteria specifically food born bacteria [27, 28]. Detha and Datta [28] indicated that Sopi and Moke, two traditional wines in Indonesia, have antimicrobial effects on E. coli and Salmonella sp., both Gram-negative bacteria.

5. Conclusion

The results obtained in this study showed that LAB, used as single starter cultures in the sorghum wort for fermentations, grew increasing organic acids and titratable acid and dropping pH. But, L. fermentum strains S6, S2 and S45, P. acidilactici strains S7, S52 and P. pentosaceus strain S5 quickly acidified the sorghum wort. These bacteria have used to produce sweet wort and tchapalo similar to those of brewer obtained by spontaneous fermentation. These beverages were able to inhibit the growth of Escherichia coli, Salmonella typhimurium and Staphylococcus aureus. Further research will focus on organoleptic properties of trial fermentations with these LAB in co-cultures. Bacteria with good fermentation behaviour will be selected and used to produce a starter cultures for sweet wort and tchapalo commercial production and thereby to improve their safety and consumer acceptability of these products.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.
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