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(RESEARCH ARTICLE)

Evaluation of potential lactic acid bacteria single starter cultures for production of traditional Ivoirian 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 Ivoirian 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 S_6, S_{42} and S_{45}, *P. acidilactici* strains S_7 and S_{52} and *P. pentosaceus* strain S_5 accelerated fermentation by dropping of ΔpH to ≥ 1.5 units and increasing of ΔAT to ≥ 0.15%. *Lactobacillus fermentum* strain S_6 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

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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 nutriment 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 1×10^6 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.

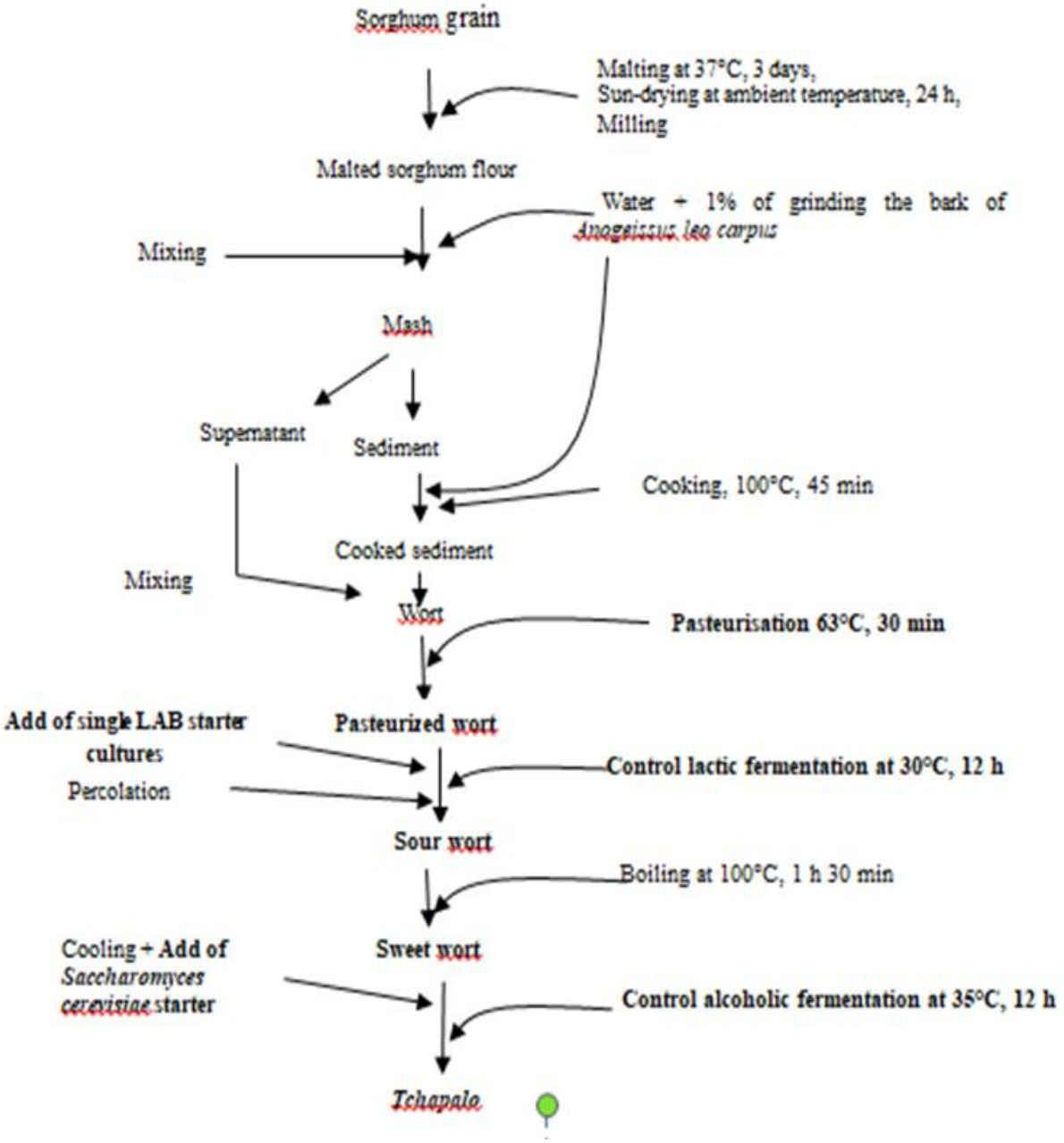

Figure 1 Diagram of *tchapalo* process in the laboratory using single starter cultures

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, Japon). 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

Cartritge-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 H_2SO_4) used at a flow rate of 0.8 mL/min was filtered through a 0.45 µm Millipore membrane filter (Sartorius AG, Goëttingen, 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 ×10^6 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. Within 6 h of fermentation, out of twenty-three bacteria, only six (26.08%) of strains namely *L. fermentum* strains S_6, S_{42} and S_{45}, *P. acidilactici* strains S_7 and S_{52} and *P. pentosaceus* strain S_5 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 S_6, S_8, S_9, S_{11}, S_{13}, S_{41}, S_{42} and S_{45} 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 S_6, S_{42} and S_{45} attained a ΔpH to ≥ 2.1 units and the increasing of ΔAT to ≥ 0.3%. However, *P. acidilactici* strain S_5 decreased ΔpH to 2.03 units and increased ΔAT to 0.33%. *Pediococcus acidilactici* strain S_7 increased ΔAT to 0.35%; while, the decrease of ΔpH of *P. pentosaceus* strains S_5 and S_{47} 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%.

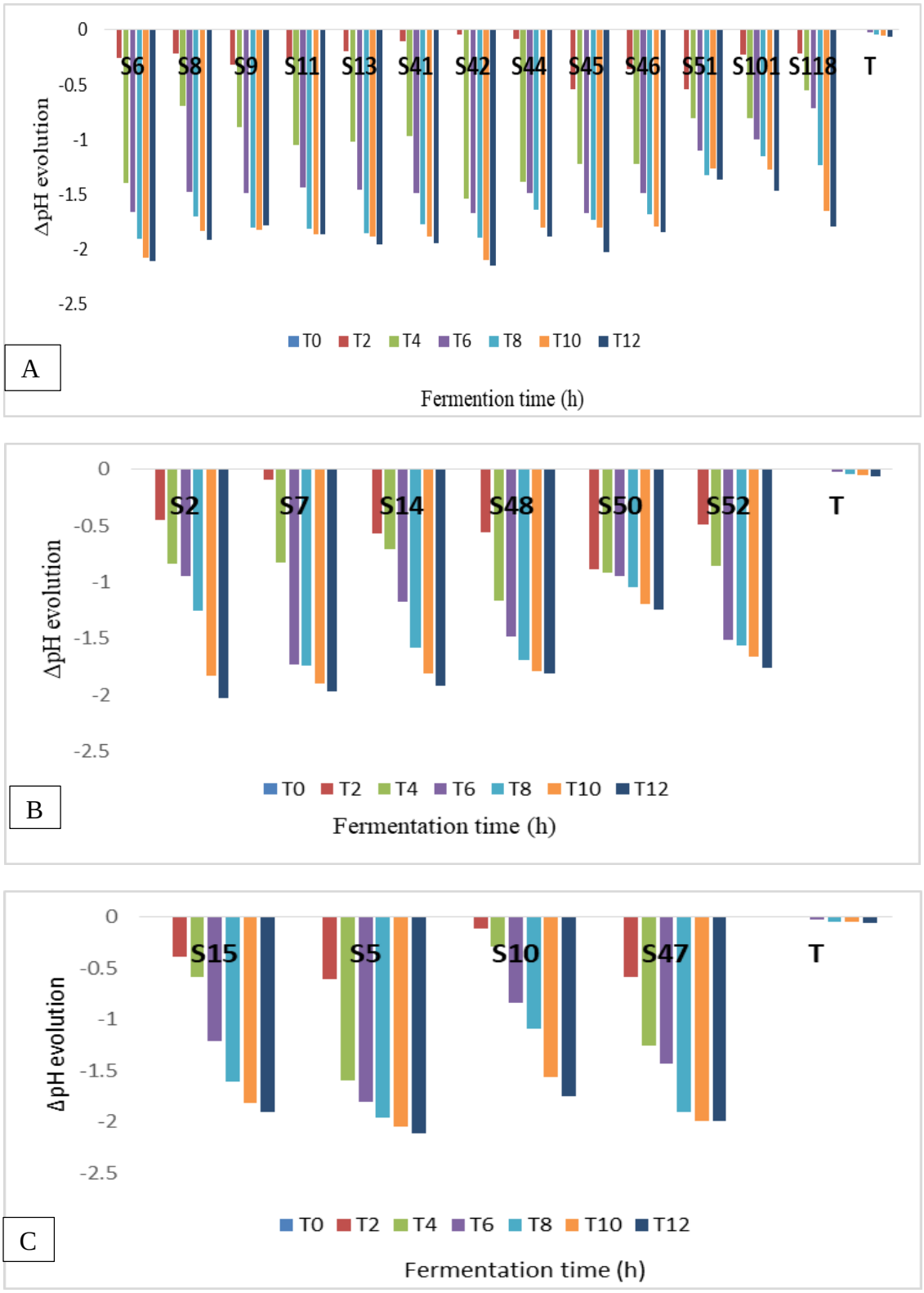

Figure 2 Changes in pH during sorghum wort fermentations using single starter cultures as inoculum. A = *Lactobacillus fermentum* strains; B = *Pediococcus acidilactici* strains; C = *Pediococcus pentosaceus* and *Lactobacillus plantarum* strains.

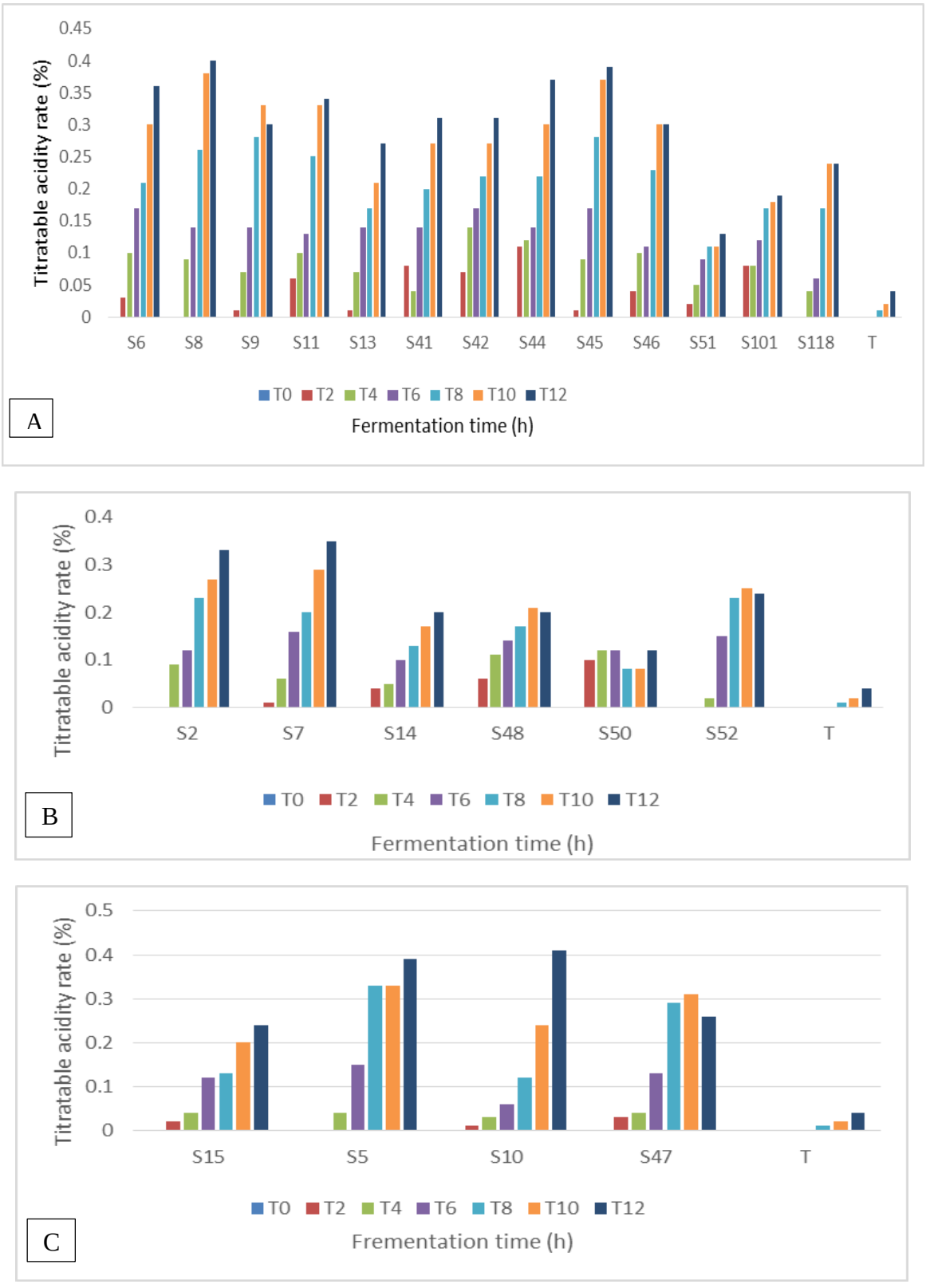

Figure 3 Changes in titratable acidity during sorghum wort fermentations using single starter cultures as inoculum. A = *Lactobacillus fermentum* strains; B = *Pediococcus acidilactici* strains; C = *Pediococcus pentosaceus* and *Lactobacillus plantarum* strains.

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).

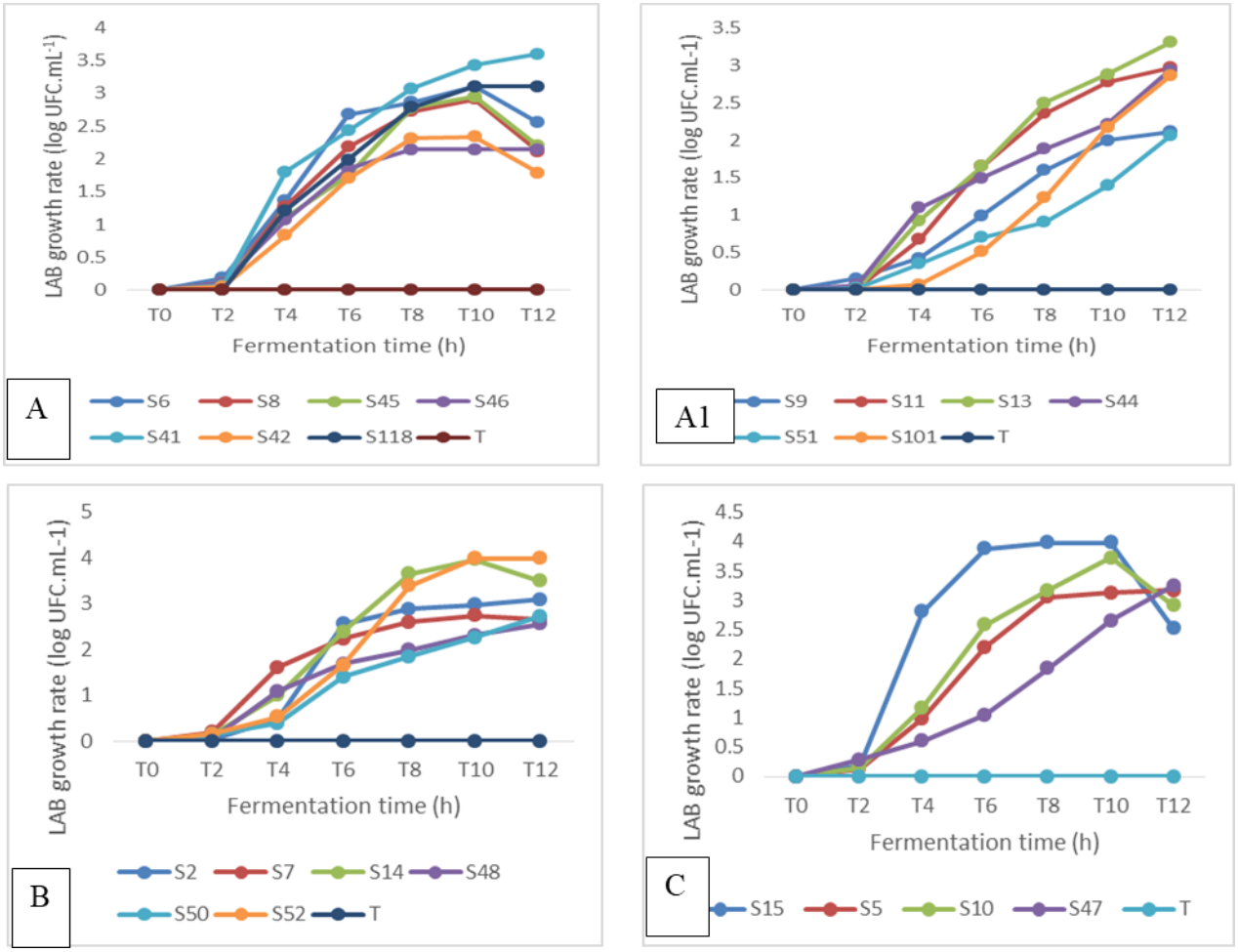

Figure 4 Growth rate of LAB species during sorghum wort fermentations using single starter cultures. A1 = *Lactobacillus fermentum* strains group 1; A2 = *Lactobacillus fermentum* strains group 2; B = *Pediococcus acidilactici* strains; C = *Pediococcus pentosaceus* and *Lactobacillus plantarum* strains.

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 S_6, S_{42} and S_{45}, *P. acidilactici* strains S_7, S_{52} and *P. pentosaceus* strain S_5 as single starter cultures were shown in Tables 1, 2 and 3. All pH of sour wort from fermentations with *L. fermentum* strains S_6, S_{42} and S_{45}, *P. acidilactici* strains S_7, S_{52} and *P. pentosaceus* strain S_5 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 S_6 and S_{45} 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 S_7 (13.0±1.4 °Brix, Table 2); while the lowest content of soluble solids was observed in sweet wort fermented with *P. acidilactici* strain S_{52} (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* S_6 (4.5±0.7 °Brix), *P. acidilactici* S_7 (5.0±0,0 °Brix), *L. fermentum* S_{45} (4.3±0.4 °Brix) and *P. acidilactici* S_{52} (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* S_5 (4.01±0.52 g/L), *P. acidilactici* S_7 (3.81±0.03 g/L) and *L. fermentum* S_{42} (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* S_6 (9.74±0.98 g/L), *L. fermentum* S_{42} (9.46±0.23 g/L) and *P. pentosaceus* S_5 (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 sweet 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 sweet wort from *P. acidilactici* strain S_7 (51.73±0.02 g/L) was highest and significantly different ($P < 0.05$) of those of brewer spontaneous fermented sweet wort (19.78±0.93 g/L) and the all others sweet 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 sweet wort from which they were derived. Thus, the lowest lactic acid content was observed in the *tchapalo* from *L. fermentum* strains S_{45} (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								
	Single starter cultures						Controls		
	S_5	S_6	S_7	S_{42}	S_{45}	S_{52}	T_F	T_L	TB
pH	3.6±0.3[a]	3.9±0.3[a]	3.5±0.1[a]	3.8±0.4[a]	4.0±0.8[a]	3.7±0.1[a]	3.7±0.6[a]	3.7±0.8[a]	6.6±0.0[c]
AT (% lactic acid)	0.3±0.1[a]	0.4±0.1[a]	0.2±0.0[a]	0.3±0.1[a]	0.4±0.1[a]	0.3±0.1[a]	0.2±0.1[a]	0.2±0.1[a]	0.1±0.0
TSS (°Brix)	5.0±0.0[a]	5.0±0.0[a]	5.5±0.7[a]	5.0±0.0[a]	5.0±0.0[a]	5.5±0.7[a]	6.0±1.4[a]	5.0±0.0[a]	5.0±0.0[a]
Tartaric acid (g/L)	4.01±0.52[c]	1.44±0.06[b]	3.81±0.03[c]	3.68±0.32[c]	1.36±0.4[b]	2.36±0.23[b]	0.67±0.24[a]	2.29±0.17[b]	1.92±0.44[b]
Acetic acid (mg/L)	0	0.06±0.01[a]	0	0.07±0.01[a]	0.06±0.01[a]	0	0	0.17±0.03[a]	0
Lactic acid (g/L)	8.12±0.07[b]	9.74±0.98[b]	7.57±0.99b	9.46±0.23[b]	7.39±0.74[b]	7.28±0.24[b]	7.85±0.5[b]	7.30±0.16[b]	0.86±0.01[b]
Fumaric acid (mg/L)	0.58±0.32[a]	4.99±0.04[c]	0	3.7±0.02[b]	0.43±0.03[a]	0	0.37±0.05[a]	4.73±0.34[c]	4.4±1.1[bc]

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_6, S_{42}, S_{45}: *L. fermentum* strains, S_7, S_{52}: *P. acidilactici* strains, S_5: *P. pentosaceus* strain

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

Characteristics	Sweet wort								
	Single starter cultures						Controls		
	S_5	S_6	S_7	S_{42}	S_{45}	S_{52}	T_F	T_L	TB
pH	3.3±0.3[a]	3.9±0.3[a]	3.8±0.2[a]	3.8±0.1[a]	3.9±0.1[a]	3.4±0.4[a]	3.5±0.1[a]	3.9±1.2[a]	6.6±0.0[c]
AT (% lactic acid)	0.3±0.1[a]	0.3±0.1[a]	0.3±0.1[a]	0.3±0.2[a]	0.3±0.1[a]	0.3±0.1[a]	0.3±0.1[a]	0.3±0.1[a]	0.1±0.0
TSS (°Brix)	11.5±2.1[b]	12.3±1.8[b]	13.0±1.4[b]	11.1±1.3[b]	11.8±3.2[b]	10.5±0.7[b]	12.2±1.7[b]	14.0±2.1[b]	5.0±0.0[a]
Tartaric acid (g/L)	6.88±0.22[c]	5.58±0.47[c]	12.13±2.12[d]	12.07±0.44[d]	6.97±1.1[c]	6.28±1.32[c]	2.38±0.37[b]	6.97±1.64[c]	1.92±0.44[a]
Acetic acid (mg/L)	0	0.15±0.05[a]	0	0.16±0.02[a]	0.17±0.02[a]	0	0	1.1±0.03[b]	0
Lactic acid (g/L)	35.93±0.23[f]	18.59±0.1[c]	51.73±0.02[g]	23.16±0.33[d]	9.79±0.03[b]	30.69±0.01[e]	19.78±0.93[d]	9.69±0.01[b]	0.86±0.01[a]
Fumaric acid (mg/L)	6.42±0.18[c]	7.17±0.1[d]	0.07±0.02[a]	8.72±1.08[e]	4.73±0.2[b]	0.77±0.11[a]	4.94±0.32[b]	6.48±0.4[c]	4.4±1.1[b]

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_6, S_{42}, S_{45}: *L. fermentum* strains, S_7, S_{52}: *P. acidilactici* strains, S_5: *P. pentosaceus* strain

Table 3 Biochemical characteristics of *tchapalo* from sorghum wort fermentations using single starter cultures

Characteristics	***Tchapalo***								
	Single starter cultures						**Controls**		
	S_5	S_6	S_7	S_{42}	S_{45}	S_{52}	T_F	T_L	TB
pH	3.9±0.3^{ab}	4.0±0.1^{ab}	3.8±0.3^{ab}	3.9±0.1^{ab}	3.9±0.1^{ab}	3.8±0.0^{ab}	3.7±0.4^{a}	4.5±0.5^{b}	6.6±0.0^{c}
AT (% lactic acid)	0.4±0.1^{a}	0.4±0.1^{a}	0.4±0.1^{a}	0.4±0.0^{a}	0.4±0.0^{a}	0.4±0.1^{a}	0.4±0.1^{a}	0.3±0.1^{a}	0.1±0.0
TSS (°Brix)	3.3±0.4^{a}	4.5±0.7^{ab}	5.0±0.0^{ab}	3.5±0.7^{a}	4.3±0.4^{ab}	4.3±1.8^{ab}	5.5±0.7^{b}	6.0±0.0^{b}	5.0±0.0^{ab}
Tartaric acid (g/L)	3.97±1.08^{b}	4.91±0.8^{b}	13.29±0.23^{d}	9.01±0.84^{c}	3.76±0.41^{b}	4.78±0.08^{b}	0	3.56±0.49^{b}	1.92±0.44^{a}
Acetic acid (mg/L)	0	0	0	0	0	0	0	0,00	0
Lactic acid (g/L)	16.94±0.07^{de}	14.27±0.14^{d}	17.24±1.22^{e}	11.83±3.32^{c}	9.77±0.23^{b}	13.79±0.96^{cd}	11.61±0.28^{bc}	9.67±0.25^{b}	0.86±0.01^{a}
Fumaric acid (mg/L)	11.97±0.67^{e}	0	0	4.76±0.47^{b}	6.29±1.02^{c}	0.67±0.34^{a}	8.25±0.94^{d}	6.29±1.2^{c}	4.4±1.1^{b}

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_6, S_{42}, S_{45}: *L. fermentum* strains, S_7, S_{52}: *P. acidilactici* strains, S_5: *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* S_7, *L. fermentum* S_{45} and *P. acidilactici* S_{52} 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		
		S_5	S_6	S_7	S_{42}	S_{45}	S_{52}	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	14±1	0	15±1	15±0	16±1	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

S_6, S_{42}, S_{45}: *L. fermentum* strains, S_7, S_{52}: *P. acidilactici* strains, S_5: *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 [13-16]. 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 S_6, S_{42} and S_{45}, *P. acidilactici* strains S_7 and S_{52} and *P. pentosaceus* strain S_5 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* S_{15} achieved its exponential phase, but did not quickly acidify the sorghum wort unlike *L fermentum* S_6 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* S_2 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 to 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* S_6, *L. fermentum* S_{45}, *P. acidilactici* S_7 and *P. acidilactici* S_{52} 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 of 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 S_6, S_{42} and S_{45}, *P. acidilactici* strains S_7, S_{52} and *P. pentosaceus* strain S_5 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.

References

[1] Aka S, N'guessan KF, Nanga YZ, Loukou YG and Dje KM. (2008). Evaluation of organic acids and sugars contents during the production of tchapalo, a traditional sorghum beer in Côte d'Ivoire. Journal of Food Technology, 6(5), 189-195.

[2] N'guessan KF, Aka S, Djeni NT and Dje KM. (2008). Effect of traditional starter inoculation rate on sorghum beer quality. Journal of Food Technology, 6 (6), 271-277.

[3] Dje KM, Aka S, Nanga YZ, Yao KC and Loukou YG. (2009). Predominant lactic acid bacteria involved in the spontaneous fermentation step of tchapalo process, a traditional sorghum beer of Côte d'Ivoire. Research Journal of Biological Sciences, 4 (7), 789-795.

[4] Kouame KB, Koko AC, Masse D and Assidjo NE. (2015). Batch fermentation process of sorghum wort modeling by artificial neural network. European Science Journal, 11, 75-93.

[5] Amane DN, Kouamé KB, Kouamé C and Assidjo EN. (2012). Optimisation du procédé de fabrication du tchapalo, bière traditionnelle ivoirienne par le plan factoriel fractionné. Afrique Science, 8(3), 69-81.

[6] Aka S, Fokou G, Doumbia M, Ahoussi JMS, Dje MK and Bonfoh B. (2017). Production et consommation du tchapalo à Abidjan: typologie des brasseuses et des consommateurs. European Scientific Journal, 13(1), 148-163.

[7] Holzapfel WH. (2002). Appropriate starter culture technologies for small- scale fermentation in developing countries. International Journal of Food Microbiology. 75, 197 –212.

[8] Aka-Gbezo S, N'Guessan KF, Yapo AE, Dje KM and Bonfoh B. (2015). Antibacterial and probiotic properties of lactic acid bacteria from traditional sorghum beer production in Côte d'Ivoire. International Journal of Advances in Pharmacy, Biology and Chemistry, 4(1), 103-113.

[9] N'guessan KF, N'dri YD, Camara F and Dje KM. (2010). Saccharomyces cerevisiae and Candida tropicalis as starter cultures for the alcoholic fermentation of tchapalo, a traditional sorghum beer. World Journal of Microbiology and Biotechnology, 26 (4), 693-699.

[10] Ayad EHE, Nashat S, El-Sadek N, Metwaly H and El-Soda M. (2004). Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food Microbiology, 21, 715-725.

[11] Arici M, Bilgin B, Sagdic O and Ozdemir C. (2004).Some characteristics of Lactobacillus isolates from infant faeces. Food Microbiology 21 (1), 19-24.

[12] Soro-Yao AA, Aka S, Thonart P and Dje KM. (2014). Assessment of the potential of lactic acid bacteria as dried starter culture for cereal fermentation. The Open Biotechnology Journal, 8, 1-5.

[13] Mukisa IM, Ntaate D and Byakika S. (2017). Application of starter cultures in the production of Enturire – a traditional sorghum-based alcoholic beverage. Food Science and nutrition, 5, 609–616.

[14] Apaliya MT, Kwaw E, Tchabo W, Sackey AS and Boateng NAS. (2017).The use of lactic acid bacteria as starter culture and its effect on the proximate composition and sensory acceptability of millet beverage. International Journal of Innovative Food Science and Technology, 1, 1-8.

[15] Olaoye OA, Ndife J and Raymond VI. (2017). Use of Lactobacillus plantarum as starter culture and its influence on physicochemical, microbiological and sensory characteristics of kunnu-aya produced from sorghum and tigernut. Journal of Food Quality, 1-8.

[16] Hassen Y, Mukisa IM, Kurabachew H and Desalegn BB. (2018). Evaluation of east and lactic acid bacteria starter cultures for the production of rice injera. Food Processing and Technology, 9(3), 1-5.

[17] Sawadogo-Lingani H, Diawara B, Traore AS and Jakobsen M. (2008). Technological properties of Lactobacillus fermentum involved in the processing of dolo and pito, West African sorghum beers, for the selection of starter cultures. Journal of Applied Microbiology, 104(3), 873-82.

[18] Mugula JK, Narvhus JA and Sørhaug T. (2003). Use of starter cultures of lactic acid bacteria and yeasts in the preparation of togwa, a Tanzanian fermented food. International Journal of Food Microbiology, 83, 307-318.

[19] Muyanja CMBK, Langsrud T and Narvhus JA. (2004).The use of starter cultures in the fermentation of bushera: a Ugandan traditional fermented sorghum beverage. Uganda Journal of Agricultural Sciences, 9, 606-616.

[20] Agarry OO, Nkama I and Akoma O. (2010). Production of Kunun-zaki (A Nigerian fermented cereal beverage) using starter culture. International Research Journal of Microbiology, 1(2), 018-025.

[21] Vieira-Dalodé G, Madodé YE, Hounhouigan J, Jespersen L and Jakobsen M. (2008). Use of starter cultures of lactic acid bacteria and yeasts as inoculum enrichment for the production of gowé, a sour beverage from Benin. African Journal of Microbiology Research, 2, 179-186.

[22] Sawadogo-Lingani H, Lei V, Diawara B, Nielsen DS, Møller PL, Traoré AS and Jakobsen M. (2007). The biodiversity of predominant lactic acid bacteria in dolo and pito wort for the production of sorghum beer. Journal of Applied Microbiology, 103(4), 765-777.

[23] Maoura N, Mbaiguinam M, Gaillardin C and Pourquie J. (2006). Suivi technique, analytique et microbiologique de la bili bili, bière traditionnelle tchadienne. Afrique Science, 20(1), 69-82.

[24] Dje KM, N'guessan KF, Djeni NT and Dadie AT. (2008). Biochemical changes during alcoholic fermentation in the production of tchapalo, a traditional sorghum beer. International Journal of Food Engineering, 4 (7), 2.

[25] Akabanda F, Kwarteng JO, Tano-Debrah K, Parkouda C and Jespersen L. (2014). The use of lactic acid bacteria starter culture in the production of nunu, a spontaneously fermented milk product in Ghana. International Journal of Food Science, 1-11.

[26] Byakika S, Mukisa IM, Mugabi R and Muyanja C. (2019). Antimicrobial activity of lactic acid bacteria starters against acid tolerant, antibiotic resistant, and potentially virulent E. coli isolated from a fermented sorghum-millet beverage. International Journal of Microbiology, 1-10.

[27] Velićanski AS, Cvetković DD, Markov SL, Šaponjac VTT and Vulić JJ. (2014). Antioxidant and antibacterial activity of the beverage obtained by fermentation of sweetened lemon balm (melissa offi cinalis l.) tea with symbiotic consortium of bacteria and yeasts. Food Technology and Biotechnology, 52 (4), 420-429.

[28] Detha AIR and Datta FU. (2016). Antimicrobial activity of traditional wines (Sopi and Moke) against Salmonella sp. and Escherichia coli. Journal of Advanced Veterinary and Animal Research, 3(3), 282-285.

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