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Use of Lactic Acid Bacteria as Starter Cultures in the Production of Tchapalo, a Traditional Sorghum Beer from Côte d'Ivoire

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Abstract

Tchapalo is an alcoholic sorghum beverage traditionally produced by spontaneous fermentation and its quality and safety are not guarantee because non-desirable flora can grow in the final product. Thus, use of pure starter cultures is necessary. This study evaluated the potential as a starter of six lactic acid bacteria strains isolated during the spontaneous fermentation in the production of tchapalo. Titratable acidity, pH, Total soluble solids, organic acids, sugars and antibacterial activity were determined. Titratable acidity, pH, Total soluble solids of wort were similar in spontaneous fermentation and fermentation with single lactic acid bacteria starter culture. However, differences were observed in organic acids production and the use of sugars. The lactic acid, the main organic acid, showed the largest amount in spontaneously fermented wort (1.92 mg/mL) followed by single culture fermented wort with the three *Lactobacillus fermentum* strains and Pediococcus acidilactici S7 (between 1.51 to 1.75 mg/mL). Acetic acid was detected in fermentations with *Lb. fermentum* strains and in spontaneous fermentation. *Pediococcus pentosaceus* S5 and *Lb. fermentum* S42 possessed a superior ability to use sugars. The low presence of acidic compounds (lactic and acetic acids) characterized tchapalo produced from fermented wort with *P. acidilactici* strains. From the examined antibacterial activity against pathogenic strains of tchapalo produced with single culture fermented wort, the absence of inhibiting capability against *S. typhi* was revealed. The six lactic acid bacteria strains could be regarded as a potential starter for tchapalo fermentation.

Keywords

Tchapalo, Starter, Lactic Acid Bacteria, Wort, Antibacterial Activity

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1. Introduction

In many African countries, fermented alcoholic beverages have a role in social functions such as marriage, naming and rain making ceremonies and constitute a source of economic return for the producers. The most African alcoholic beverages are opaque beers often produced from sorghum [1, 2]. These beers are known as tchoukoutou in Benin, dolo in Burkina-Faso, pito in Ghana, Burukutu, otika or sekete in Nigeria; impeke in Burundi and tchapalo in Côte d'Ivoire [3, 4].

In Côte d'Ivoire, tchapalo is a popular beverage made from red sorghum malt. The beer is opaque, with a red color, an alcohol content of 3% to 5% v/v, total soluble solids of 8 to 9◦Brix, and a pH of 3 to 4 [5, 6]. The traditional process of tchapalo production involves a series of stages such as malting and mashing the grain, sedimentation, boiling the colloidal suspension, mixing the suspension with the supernatant liquor, souring (spontaneous lactic acid

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fermentation), boiling the cooling, and pitching the cooled wort with dried yeast harvested from previous brews for alcoholic fermentation [7]. The fermentation provides a natural way to destroy undesirable components, to enhance the nutritive value and appearance of the food. It has also the potential of enhancing foods safety by controlling the growth and multiplication of number of pathogens in foods [8]. However, this fermentation is spontaneous and the microorganisms responsible are the microbiota indigenously present on the substrate, the raw materials, equipment and local environments [3]. Due to this uncontrolled fermentation, the final beverage has a poor microbiological safety. In addition, the quality of beer varies often from one production to another. According to Djè et al. [9], the control of this fermentation step is very important because it determines the further to process, organoleptic properties and the preservation of the sweet wort.

The use of starter cultures was proposed like 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 safety in African traditional fermented beverages [10, 11]. Different species of lactic acid bacteria (LAB) have been used successfully as starter cultures to ferment traditional products from cereals beverages through the use of competitive or antagonistic microorganisms or their metabolic products, to prevent or inhibit the growth of undesired microorganisms into the African traditional fermentation food [12, 13, 14]. LAB involved in the spontaneous fermentation step processing of tchapalo were identified. The main species belong to the genus *Lactobacillus*, *Enterococcus*, *Pediococcus* and *Leuconostoc* [9, 15]. Previously, Aka-Gbézo et al. [16] reported that strains of *P. acidilactici* isolated during tchapalo processing inhibited the growth of spoilage and food borne opportunistic pathogens and could be used as potential biopreservative starter cultures to produce sweet wort, tchapalo and other beverages.

Therefore, the present study was conducted to evaluate the potential as starter of six LAB strains isolated during the spontaneous fermentation in the production of tchapalo in order to alleviate the problems of variations in organoleptic quality and microbiological safety.

2. Material and Methods

2.1. Samples Collection

Red sorghum grains that were used for sorghum wort production and samples (250 mL) of fresh tchapalo and sour wort were collected from traditional brewers at Abobo Pk18 (Abidjan, Southern Côte d'Ivoire). Fresh tchapalo and sour wort were used as the references (spontaneous fermentation) for samples that were prepared in the laboratory. All experiments were carried out independently in triplicate.

2.2. Strains and Growth Condition

Three strains of *Lb. fermentum* (S6, S42 and S45), two of *P. acidilactici*: (S7 and S52) and one of *P. pentosaceus*: (S5) isolated during the spontaneous fermentation of tchapalo in a previous study (Aka-Gbézo [17] were used in single culture for the production of tchapalo. LAB strains were grown on MRS agar for 48 h at 37°C. An isolated colony was picked and subcultured in MRS broth at 37°C for overnight. After growth, each LAB culture was centrifuged at 4000 x g for 10 min and the pellet was collected for wort inoculation.

E. coli ATCC 25922, *S. typhi* ATCC 14028 and *S. aureus* 25923 were used as foodborne pathogens. They were subcultured at 37°C in nutrient broth, after growing on nutriment agar.

All these strains were obtained from the Laboratory of Food Biotechnology and Microbiology, faculty of food sciences and technologies, Nangui Abrogoua University, Côte d'Ivoire.

2.3. Malting of Sorghum

Red sorghum grains (5 Kg) obtained from traditional brewer were sorted manually to remove debris. Then, the grains selected were steeped in water (20 L) at 37°C for 18 h. After steeping, the grains were drained and were germinated at 37°C for 3 days before drying at ambient temperature for 24 h. The dried grains were mashed with breaker.

2.4. Production of Sour Wort

Wort was produced by decantation mashing described by Aka[7]. Briefly, 3.4 Kg of milled sorghum malt (99% sorghum and 1% of grinding the bark of *Anogeissus leo carpus* were mixed with 20 L water at 37°C and then left in decantation during 45 min. Thereafter, 10 L of the supernatant were removed while the sediment was heated at 100°C for 1 h 30 min to gelatinize malt starch. The supernatant was precooked at 45°C and then was mixed with the boiled sediment. Finally, the wort obtained was pasteurized at 63°C for 30 min and LAB strains were inoculated with 1×10^6 cfu mL^{-1} and incubated for 12 h at 37°C. After that, sour wort was obtained. Every 2 h, 15 ml was sampled under aseptic conditions for physico-chemical analysis. Three trial fermentations were realized.

2.5. Fermentation

The sour wort was boiled during 6 h to obtain sweet wort. Then, dried yeast harvested from traditional producers previous brew was inoculated to sweet wort at 1% (w/v) for

alcoholic fermentation during 12 h. The product obtained after alcoholic fermentation was tchapalo.

2.6. Wort and Beer Analysis

2.6.1. Physico-chemical Analysis

The pH was measured using a digital pH-meter (P107 Consort). The Total Titratable Acidity (TTA), expressed as a percentage lactic acid, was determined by titrating 5 mL of the sample against 0.1 N (NaOH) using phenolphthalein as the indicator. The Total Soluble Solids (TSS) content, expressed as °Brix value, was determined in each sample using a hand refractometer.

2.6.2. Organic Acids and Sugar Analysis

Organic acids and sugar contents were analyzed by high-performance liquid chromatography, according to the method described by N'guessan [18]. Analysis was carried out using the HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with a pump (Shimadzu LC-6A Liquid Chromatograph), a detector (Shimadzu SPD-6A UV Spectrophotometric detector), and an integrator (Shimadzu C-R 6A Chromatopac). The samples were firstly centrifuged at 3000 rpm for 20 min. Then, they were filtered through 0.20 µM pore size filters (Corning syringe filters, Sigma-Aldrich, Germany). Chromatographic separation was performed using an ion-exclusion ORH-801 column (300 × 9 × 6.5 mm, Interchrom, Paris, France) and column oven (Interchrom) set to 37°C. The mobile phase was 0.004 N H_2SO_4 with a flow rate of 0.8 mL/min, and detection at 210 nm was used. Identifications were based on matching retention times of standards for sugar (fructose, glucose, sucrose and maltose) and for organic acids (tannic, citric, tartaric, lactic acid, acetic acid, fumaric acid and propionic acid) which were purchased respectively from Merck (Merck Co., Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich GmbH, Seelze, Germany). Each sample was injected in duplicate. Organic acids were identified by comparing their retention times with those of standards.

2.7. Antibacterial Activity of Wort Supernatant

Antibacterial activity was assayed using an agar diffusion method described by Arici et al. [19]. Ten (10) mL of sour wort and tchapalo resulting from inoculation of each LAB strain were centrifuged (4000 × g, TGL-16 M) for 10 min at 4°C. 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 10^6 CFU/mL were spread on solid Mueller-Hinton Medium. Filtered sour wort and *tchapalo* 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 for 24 h at 37°C. 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 double.

2.8. Statistical Analysis

The statistical analysis was performed using XLSTAT 2017 (Addinsoft, New York, USA) for Microsoft Excel. The one-way ANOVA analysis, follow by the Tukey's test was used to compare the diameters of inhibition and the variables analyzed on the sour wort and tchapalo. Differences were considered significant at level $p < 0.05$. Principal component analysis (PCA) was used to compare wort or tchapalo samples obtained from different LAB strains.

3. Results

3.1. Contribution of LAB Strains in the Production of Sour Wort

3.1.1. Physicochemical Properties

Total titrable acidity and pH of sorghum wort were significant difference than that found in wort sour as shown in table 1. However, there was no significant difference ($P > 0.05$) in comparing spontaneous fermentation pH and total titrable acidity within the six LAB strains. The pH values were between 3.50 and 4.00 in single fermentation and 3.65 in spontaneous fermentation. The total titrable acidity was 0.23% for spontaneous fermentation and ranged from 0.23 to 0.39% in the laboratory sour wort with each LAB strain. The total soluble solids of the experimental and spontaneous fermentation samples was in the same range. The value ranged from 5.00 to 5.50 °Brix and were not significantly different ($P > 0.05$) with the control (sorghum wort).

Table 1. Effect of lactic acid bacteria on the pH, total titratable acidity and total soluble solids of sorghum wort.

	Sorghum wort	***Lb. fermentum* S6**	***Lb. fermentum* S42**	***Lb. fermentum* S45**	***P. acidilactici* S7**	***P. acidilactici* S52**	***P. pentosaceus* S5**	**Spontaneous fermentation**
pH	6.65±0.01[b]	3.90±0.28[a]	3.80±0.42[a]	4.00±0.85[a]	3.50±0.14[a]	3.65±0.07[a]	3.60±0.28[a]	3.65±0.64[a]
Total titrable acidity (%)	0.12±0.01[b]	0.36±0.03[a]	0.32±0.16[a]	0.39±0.09[a]	0.23±0.01[a]	0.30±0.09[a]	0.32±0.12[a]	0.23±0.04[a]
Total soluble solids (°Brix)	6.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.50±0.71[a]	5.50±0.71[a]	5.00±0.00[a]	5.00±0.00[a]

The values are the means of three independent samples ±standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$).

3.1.2. Organic Acid and Sugar Contents

As shown in table 2, organic acid and sugar contents of sour wort were affected by the presence of LAB. Tannic, lactic and propionic acid concentrations increased in the starters and spontaneous fermentation. There was no a significant difference in the production of tannic and propionic acids in all the sours wort. Lactic acid was the major compound produced during sour wort production. The largest amount of lactic acid was produced in spontaneously fermented wort (1.92 mg/mL) followed by single culture fermented wort with the three *Lb. fermentum* strains and *P. acidilactici* S7 (between 1.51 to 1.75 mg/mL). Citric acid with the initial value 0.49 mg/mL decreased to undetectable level after all fermentations. Acetic acid was detected in fermentations with *Lb. fermentum* strains and in spontaneous fermentation.

Fructose, glucose, saccharose and maltose were sugars detected in sorghum wort. Among these sugars, saccharose was detected in spontaneous fermentation and single culture fermented wort with *P. pentosaceus* S5 and *Lb. fermentum* S42. In these samples, saccharose concentration was reduced from 0.49 mg/mL to concentrations 0.24 to 0.09 mg/mL. Glucose content increased in the sour wort obtained with *Lb. fermentum* S6, *Lb. fermentum* S45 and *P. acidilactici* S7. The largest amount (3.96 mg/mL) was observed with *P. acidilactici* S7. High production of fructose (from 0.10 to 1.25 mg/mL) was observed in the fermented wort with *Lb. fermentum* S45. Maltose concentration dropped to 0.12 and 0.21 mg/mL respectively in the single fermentation with *P. acidilactici* S7 and *Lb. fermentum* S6. However, *P. acidilactici* S52 increased maltose content from 0.95 to 2.51 mg/mL.

Table 2. Effect of lactic acid bacteria strains on the organic acid and sugar contents of sorghum wort.

Compound detected (mg/mL)	Sorghum wort	*Lb. fermentum* S6	*Lb. fermentum* S42	*Lb. fermentum* S45	*P. acidilactici* S7	*P. acidilactici* S52	*P. pentosaceus* S5	Spontaneous fermentation
Organic acid contents								
Tannic acid	0.05±0.01^{b}	0.20±0.03^{a}	0.20±0.01^{a}	0.20±0.01^{a}	0.19±0.05^{a}	0.22±0.07^{a}	0.14±0.02^{a}	0.26±0.04^{a}
Oxalic acid	0.07±0.01^{a}	0.07±0.01^{a}	0.07±0.01^{a}	0.07±0.01^{a}	0.06±0.00^{a}	0.05±0.00^{a}	0.05±0.01^{a}	0.06±0.01^{a}
Citric acid	0.49±0.01	nd	nd	nd	nd	nd	nd	nd
Tartric acid	0.27±0.05^{a}	nd	nd	nd	0.12±0.05^{a}	nd	0.21±0.01^{a}	0.40±0.06^{b}
Lactic acid	0.31±0.10^{d}	1.51±0.38^{a}	1.72±0.1^{a}	1.75±0.10^{a}	1.57±0.30^{a}	1.04±0.12^{b}	1.15±0.25^{b}	1.92±0.19^{c}
Acetic acid	nd	0.27±0.01^{a}	0.27±0.03^{a}	0.30±0.05^{a}	nd	nd	nd	0.34±0.02^{a}
Fumaric acid	nd	0.02±0.00^{a}	nd	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	nd	0.03±0.00^{a}
Propionic acid	0.13±0.07^{b}	0.56±0.07^{a}	0.69±0.10^{a}	0.72±0.21^{a}	0.56±0.07^{a}	0.46±0.15^{a}	0.51±0.09^{a}	0.53±0.05^{a}
Sugar contents								
Fructose	0.10±0.05^{a}	0.15±0.03^{a}	nd	1.25±0.07^{b}	nd	0.06±0.04^{a}	nd	nd
Glucose	0.30±0.05^{c}	1.69±0.90^{a}	nd	2.21±0.08^{a}	3.96±0.10^{b}	0.30±0.05^{c}	nd	nd
Saccharose	0.49±0.03^{a}	0.59±0.07^{a}	0.15±0.05^{b}	nd	0.32±0.04^{a}	0.38±0.06^{a}	0.09±0.04^{b}	0.24±0.08^{ab}
Maltose	0.95±0.08^{c}	0.21±0.04^{a}	nd	nd	0.12±0.05^{a}	2.51±0.12^{b}	nd	nd

The values are the means of three independent samples ±standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$). nd=not detected

3.1.3. Principal Components Analysis (PCA) of Sour Wort

Principal components analysis (PCA) was used to simplify the interpretation of the data. The two main components Dim 1 and Dim 2 explained respectively 46.88% and 15.55% of the total data variance (Figure 1). The corresponding loading table (Table 3) established the importance of each parameter to the two main components. As shown in figure 1, all fermented wort was clearly differentiated from the sorghum wort, which lie in the negative region of Dim 1. The compounds that mainly contributed positively (F loading > 0.7) to Dim1 were tannic, lactic propionic acids. The pH, total soluble solids and citric acid contributed negatively to Dim 1. As for Dim 2, only maltose was observed a strong negative correlation. This indicates that fermented wort were characterized by the reduction of the pH, total soluble solids and citric acid and contrary the production of tannic, lactic and propionic acids. Depending on the variables analyzed, the wort obtained with spontaneous fermentation was intermediate between single culture fermented with *Lb. fermentum* strains on the one hand and *P. acidilactici* and *P. pentosaceus* on the other (Figure 1).

Table 3. The principal component loadings resulting from the principal component analysis of sour wort.

	Dim 1	Dim 2
pH	-0.7321	-0.2323
Total. titrable. acidity	0.6663	-0.0044
Total. soluble. solids	-0.7946	0.0040
Fructose	0.1358	-0.3472
Glucose	0.0601	-0.0253
Saccharose	0.2712	0.0223
Maltose	-0.1862	-0.8030
Tannic acid	0.7007	0.1240
Oxalic acid	0.0004	-0.3161
Citric acid	-0.8397	-0.1389
Tartric acid	-0.1436	0.0100
Lactic acid	0.8799	-0.0026
Acetic acid	0.4443	-0.2218
Fumaric acid	0.2425	0.0663
Propionic acid	0.9336	-0.0085

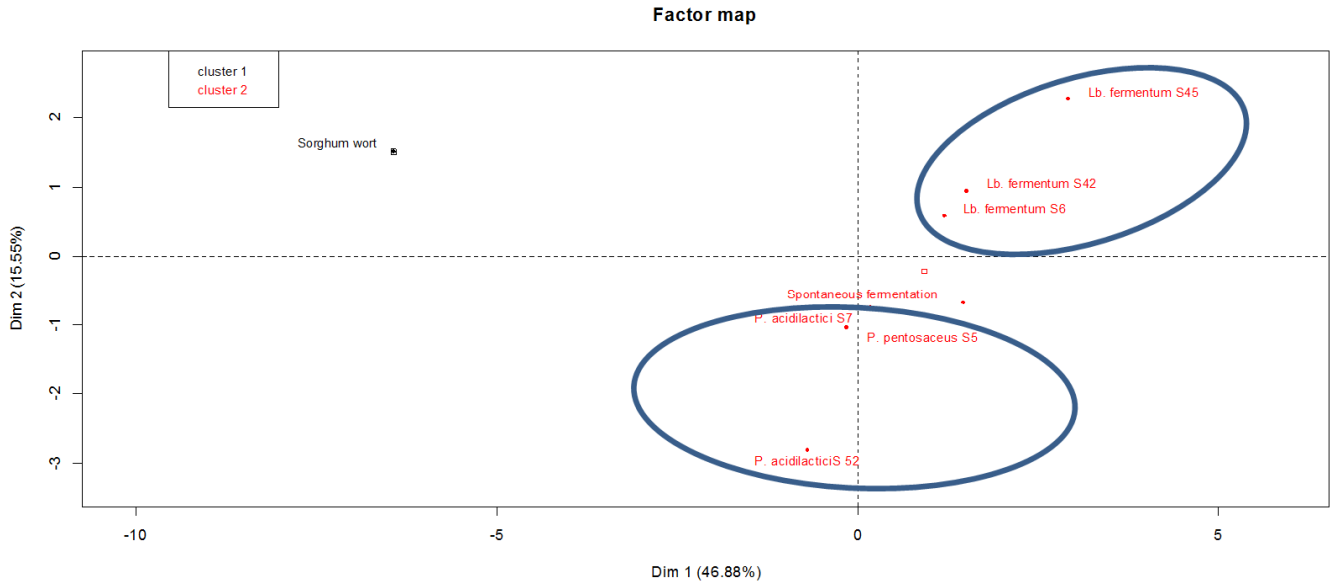

Figure 1. PCA plots of sour wort produced from LAB strains in single culture and spontaneous fermentation.

3.2. Characteristic of Tchapalo Produced with Single LAB Culture Sour Wort

3.2.1. Physicochemical Properties

Table 4 presented pH, total titrable acidity and total soluble solids of tchapalo obtained with different sour worts. Total soluble solids and titrable acidity of all tchapalo produced with sour wort fermented with single LAB strain were not significantly different ($p>0.05$) to tchapalo from spontaneous fermented wort. However, the pH showed differences. The lowest value of pH (3.65) was obtained in tchapalo from spontaneous fermented wort. For tchapalo produced with starter fermented wort, pH varied between 3.80 and 3.95.

Table 4. pH, total titratable acidity and total soluble solids of tchapalo obtained from different sour wort produced by LAB strains in single or spontaneous fermentation.

	Lb. fermentum S6	*Lb. fermentum* S42	*Lb. fermentum* S45	*P. acidilactici* S7	*P. acidilactici* S52	*P. pentosaceus* S5	Spontaneous fermentation
pH	3.95±0,07^a	3.85±0,07^a	3.90±0,14^a	3.80±0,28^a	3.80±0,00^a	3.90±0,28^a	3.65±0,35^b
Total titrable acidity (%)	0.37±0,11^a	0.38±0,03^a	0.37±0,01^a	0.39±0,09^a	0.44±0,01^a	0.41±0,06^a	0.42±0,04^a
Total soluble solids (°Brix)	4.50± 0.71^a	4.50± 0.79^a	4.25± 0.35^a	5.00± 0.00^a	4.25± 0.53^a	4.25± 0.35^a	4.5±0.82^a

The values are the means of three independent samples ±standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$).

3.2.2. Organic Acid and Sugar Contents

Organic acid contents of drink obtained from the sour wort produced with *Lb. fermentum* S6, *Lb. fermentum* S42 and *Lb. fermentum* S45 were similar to those of produced with spontaneous fermented wort except for lactic acid. Lactic acid was observed the highest amount (3.24 mg/mL) in the tchapalo produced with spontaneous fermented wort. The value ranged from 2.03 to 2.22 mg/mL for beer obtained from sour wort produced with *Lb. fermentum* strains. In the tchapalo produced with wort fermented by *Pediococcus* strains, acetic acid was not detected and lactic acid had the least content with value between 1.10 and 1.86 mg/mL.

Fructose, glucose and maltose were the sugars detected in all the tchapalo produced. Only glucose content showed a significant difference ($p>0.05$) between the samples analyzed. The tchapalo produced with spontaneous fermented wort observed lower glucose content (4.81 mg/mL). Variations were observed in the glucose content of tchapalo with the use of different LAB in single culture. The tchapalo produced from the fermentation of sorghum wort with *Lb. fermentum* S6 showed the highest glucose content (6.94 mg/mL).

Table 5. Organic acid and sugar contents of tchapalo obtained from different sour wort produced by LAB strains in single and spontaneous fermentation.

Compound detected (mg/mL)	*Lb. fermentum* S6	*Lb. fermentum* S42	*Lb. fermentum* S45	*P. acidilactici* S7	*P. acidilactici* S52	*P. pentosaceus* S5	Spontaneous fermentation
Organic acid contents							
Tannic acid	0.17±0.04^a	0.19±0.02^a	0.18±0.02^a	0.17±0.07^a	0.22±0.05^a	0.19±0.07^a	0.19±0.02^a
Tartric acid	0.39±0.01^a	0.22±0.01^a	0.40±0.03^a	1.12±0.01^b	0.76±0.05^{ab}	0.83±0.03^a	0.46±0.01^a

Compound detected (mg/mL)	*Lb. fermentum* S6	*Lb. fermentum* S42	*Lb. fermentum* S45	*P. acidilactici* S7	*P. acidilactici* S52	*P. pentosaceus* S5	Spontaneous fermentation
Lactic acid	2.21±0.71^{a}	2.22±0.55^{a}	2.03±0.27^{a}	1.10±0.66^{b}	1.86±0.42^{ab}	1.85±0.39^{ab}	3.24±0.37^{c}
Acetic acid	0.12±0.02^{a}	0.17±0.01^{a}	0.15±0.01^{a}	nd	nd	nd	0.26±0.03^{a}
Propionic acid	1.19±0.21^{a}	1.05±0.17^{a}	1.32±0.30^{a}	1.45±0.50^{a}	1.75±0.45^{a}	1.38±0.26^{a}	1.65±0.34^{a}
Sugar contents							
Fructose	4.00±0.95^{a}	3.87±0.77^{a}	3.91±0.38^{a}	3.73±0.84^{a}	3.86±1.02^{a}	3.83±0.71^{a}	3.57±0.42^{a}
Glucose	6.94±0.85^{a}	5.20±1.21^{b}	6.06±1.36^{ab}	5.59±0.62^{ab}	5.06±1.51^{b}	5.79±0.88^{ab}	4.81±1.05^{b}
Maltose	0.50±0.07^{a}	0.66±0.12^{a}	0.47±0.19^{a}	0.64±0.30^{a}	0.68±0.09^{a}	0.60±0.15^{a}	0.65±0.02^{a}

The values are the means of three independent samples ±standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$). nd=not detected

3.2.3. Principal Components Analysis of Tchapalo

The main components Dim 1 and Dim 2 of PCA of tchapalo obtained from the different sour wort explained respectively 45.28% and 26.09% of the total data variance (Figure 2). The beer produced were separated in three groups named cluster. Cluster 3 was tchapalo obtained from spontaneous fermentation of sorghum wort were differentiated to other tchapalo produced with starter fermented wort. Tchapalo produced from the sour wort obtained with *Lb. fermentum* strains, which lie in the negative region of Dim 1, were grouped in cluster 1. The tchapalo obtained from spontaneous fermentation were characterized by a lowest of the pH and glucose content according to the correlation in table 6. On contrary, this tchapalo had a highest level of total titrable acidity. The low presence of acidic compounds (lactic and acetic acids) characterized tchapalo produced from fermented wort with *P. acidilactici* strains (cluster 2), which lie in the positive region of Dim 2. Depending on the variables analyzed, the tchapalo obtained with spontaneous fermentation was intermediate between single culture fermented with *Lb. fermentum* strains on the one hand and *P. acidilactici* and *P. pentosaceus* on the other (Figure 2).

Table 6. The principal component loadings resulting from the principal component analysis of tchapalo.

	Dim 1	Dim 2
pH	-0.7739	-0.0739
Total titrable acidity	0.8038	-0.0247
Total soluble solids	0.0058	0.1142
Fructose	-0.6210	0.0247
Glucose	-0.8164	0.0227
Maltose	0.6848	0.0270
Tannic acid	0.4433	-0.0001
Tartric acid	0.1147	0.7768
Lactic acid	0.0659	-0.8511
Acetic acid	-0.0008	-0.9168
Propionic acid	0.6558	0.0378

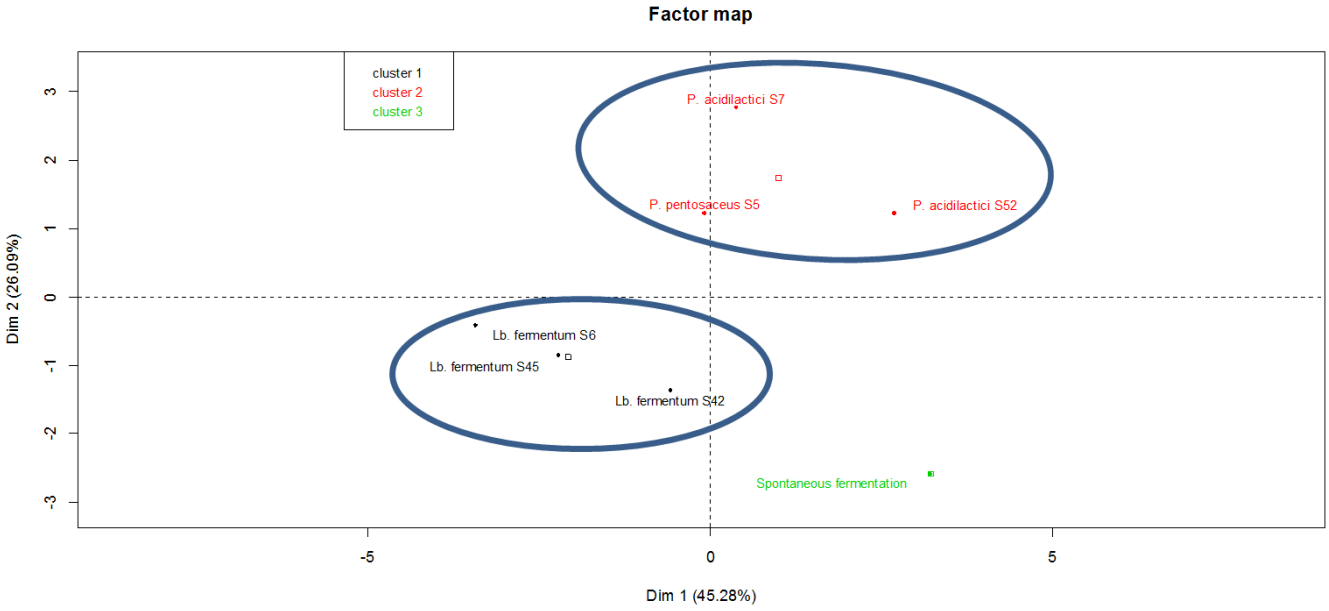

Figure 2. PCA plots of tchapalo produced with sour wort from LAB strains in single culture and spontaneous fermentation.

3.3. Antibacterial Activity of Supernatants from Tchapalo

Inhibitory spectra of *tchapalo* produced were presented in table 7. Tchapalo produced from spontaneous fermentation of sorghum wort exhibited antibacterial activity against *E. coli*, *S. typhi* and *S. aureus*. The inhibition zones diameters were 16 mm against *E. coli* and *S. typhi*, and 12 mm against *S. aureus*. From the examined tchapalo produced in single

culture fermented wort, the absence of inhibiting capability against *S. typhi* was revealed. However, it was noticed that inhibitor activity of tchapalo obtained with single culture fermented wort against *S. aureus*, except for *Lb. fermentum* S42 and *Lb. fermentum* S45, was higher than for spontaneous fermentation. Furthermore, tchapalo obtained with fermented wort by *Lb. fermentum* S45, *P. acidilactici* S7 and *P. acidilactici* S52 inhibited the growth of *E. coli* with the inhibition diameters between 14 and 15 mm.

Table 7. Diameters of inhibition (mm) of tchapalo supernatants from different sour wort against pathogenic strains.

Indicatory strains	*Lb. fermentum* S6	*Lb. fermentum* S42	*Lb. fermentum* S45	*P. acidilactici* S7	*P. acidilactici* S52	*P. pentosaceus* S5	Spontaneous fermentation	Sorghum wort
E. coli	0	0	15 ± 1^{ab}	14 ± 1^{b}	15 ± 0^{ab}	0	16 ± 1^{a}	0
S. typhi	0	0	0	0	0	0	16±1	0
St. aureus	13 ± 1^{ab}	12 ± 1^{ab}	11 ± 1^{ab}	15 ± 1^{a}	13 ± 1^{ab}	13 ± 1^{ab}	12 ± 3^{ab}	0

Value are the mean of two independent experiments by duplicate ± standard deviation. On the same line, mean values with the same letter are not significantly different ($p > 0.05$)

4. Discussion

The present study aimed to evaluate the potential of six LAB strains in the production of tchapalo, an Ivoirian traditional sorghum beer. All the strains tested in single fermentation were able to reduce pH within acidic range (between pH 3.60 and 4.00) and to increase total titrable acidity as in spontaneous fermentation. Acidification with application of starter cultures of LAB including *Lb. fermentum* has been reported in a number of studies on fermented cereal beverages [20, 13, 10]. This acidification is essential in tchapalo processing, because it contributes to the development of characteristic flavor, color and aroma, as well as to the microbiological stability of the product. Acidic conditions generally favor the growth of yeasts that ensure alcoholic fermentation[7]. It could be as a result of the organic acid produced by LAB strains. HPLC analysis revealed the decrease of tartric and citric acids and the production of lactic, tannic and propionic acids in single and spontaneous fermentation. This dropping could attributed to a microbial degradation. Among the acid compounds detected, lactic acid was the main acid produced. The lactate content of spontaneously fermented wort was 1.92 mg/mL, while that produced by different starter cultures was in the range 1.04 to 1.72 mg/mL. Muyanja et al. [21] found similar results in the use of starter culture in the production of bushera, the similar beer of tchapalo from Uganda. Acetic acid was only detected in the fermentation with *Lb. fermentum* strains and in spontaneous fermentation. The presence of acetic acid in these samples could be attributed to a shift from hetero fermentative metabolism of *Lb. fermentum* strains as mentioned by Papadelli et al. [22].

The formation of organic acids is the consequence of sugar metabolism. There was a significant difference in the sugar content of sour wort obtained with the different LAB. *Lactobacillus fermentum* S42, *P. pentosaceus* S5 and Spontaneous fermentation seemed to use glucose, maltose and fructose until undetectable level. Glucose content increased in the sour wort obtained with *Lb. fermentum* S6, *Lb. fermentum* S45 and *P. acidilactici* S7. Production of glucose could be due to ability of these LAB strains to hydrolysis starch. Nanodoum et al. [23] reported that LAB hydrolyze starch of sorghum leading to an acidified product particularly lactic acid that reduced the pH.

The sour wort obtained depend on LAB species. In fact, our results showed that sour wort produced with *Lb. fermentum* strains differ to those produced with *P. acidilactici* and *P. pentosaceus* strains. The spontaneous fermentation wort was intermerdiary wort between single culture fermented with *Lb. fermentum* strains on the one hand and *P. acidilactici* and *P. pentosaceus* on the other hand. These results could be due to a different contribution of *Lactobacillus* and *Pediococcus* species in the production of sour wort. *Lactobacillus* and *Pediococcus* species have been identified simultaneously in the spontaneous fermentation of sour wort[9]. It has also been demonstrated that each strain present may affect the quality of sorghum wort differently [21].

The tchapalo obtained from spontaneously fermented wort was different to that produced by different starter cultures. Furthermore, the tchapalo obtained by wort treated with *P. acidilactici* and *P. pentosaceus* strains was different that with *Lb. fermentum* used as starter culture. This observation in tchapalo is in accordance with the differences observed in sour wort. The tchapalo produced from wort obtained by spontaneous fermentation were characterized on the one hand by a lowest of the pH and glucose content and on the other by a highest of the lactic acid. Due to the biochemical differences, the organoleptic quality of the beer produced with wort obtained spontaneously could be different to the beer produced by single culture fermentation. Afolabi [12] reported that the amount of total residual sugar in beer influences the organoleptic properties of beer.

Tchapalo produced from spontaneous fermentation of sorghum wort exhibited antibacterial activity against all the pathogenic strains tested namely *E. coli*, *S. aureus* and *S. typhi*. For tchapalo

produced with wort fermented by single starter culture, the absence of inhibiting capability against *S. typhi* was revealed. The difference in the pathogenic strains control could be explained by the difference in rate of production of antimicrobials compounds. In fact, various factors such as low pH due to the production of organic acids (lactic and acetic acids), carbon dioxide, hydrogen peroxide, ethanol, diacetyl and bacteriocins contributing to the antimicrobial activity of LAB [24]. In this study, lactic acid observed the highest amount (3.24 mg/mL) in the tchapalo produced with spontaneous fermented wort. Ogunbanwo [25] reported that production of the primary metabolite, lactic acid and the resulting pH decrease is the main preserving factor in food fermentation.

5. Conclusion

This study showed that the LAB strains tested can be used as single strain starter cultures to produce tchapalo with an acidification similar to that of spontaneously fermented. However, spontaneously fermented tchapalo was intermediary between that produced with *Lb. fermentum* strains in the one hand and *Pediococcus acidilactici* and *P. pentosaceus* in the other hand. Furthermore, the inhibitory activity of the tchapalo produced with spontaneous fermented wort against pathogen strains was greater than that of tchapalo obtained with wort fermented by single starter culture. Thus, in order to obtain a beer with similar characteristics to those produced spontaneously, a combination of *Lb. fermentum* and *P. acidilactici* or *P. pentosaceus* strains would be suitable necessary.

Authors' Contributions

All authors contributed equally for this work. They read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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