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Biochemical and Microbiological Changes during the Ivorian Sorghum Beer Deterioration at Different Storage Temperatures

Constant K. Attchelouwa *, Solange Aka-Gbézo, Florent K. N'guessan, Clémentine A. Kouakou and Marcellin K. Djè

Laboratory of Food Biotechnology and Microbiology, Training and Research Unit in Food Science and Technology (UFR-STA), Nangui Abrogoua University, 02 BP 801 Abidjan 02, Ivory Coast; solangeakan@yahoo.fr (S.A.-G.); genie_ci@yahoo.fr (F.K.N.); kclementine24@yahoo.fr (C.A.K.); djekoffr@yahoo.fr (M.K.D.)

* Correspondence: constantattchelouwa@hotmail.fr; Tel.: +225-088-406-27; Fax: +225-203-043-00

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Abstract: In order to extend shelf life of traditional sorghum beers, it is of importance to evaluate their spoilage characteristics. Therefore, the microbiological, biochemical, and sensory changes of the Ivorian sorghum beer *tchapalo* during storage at ambient temperature (28 to 30 °C) for four days and at 4 °C for six days were assessed. The aerobic mesophilic bacteria and the yeast counts remained stable during the storage time. However, variations were observed in the lactic acid bacteria and acetic acid bacteria counts. The deteriorating *tchapalo* acidity did not show significant variations. In contrast, the total soluble solids decreased at ambient temperature and remained stable at 4 °C. Lactic acid was a major compound during storage, and acetic acid was found at a detectable level of 1.26 mg/mL after the third day at ambient temperature. The ethanol contents increased significantly at ambient temperature after two days and then decreased but showed a fair decrease at 4 °C. Evaluating the beer's appearance, odor, and taste, a panel considered the beers to be spoiled after two days when stored at 28 to 30 °C and after three days when stored at 4 °C.

Keywords: bacteria; sensory analysis; storage; temperature; tchapalo; yeasts

1. Introduction

Fermented beverages are one of the indispensable components of the dietary culture of every community in the world. More than any other fermented foodstuff, fermented beverages have served to delineate social relations between family and group members, as well as among the elite and commoners, and to express a relationship between humans and deities [1]. One of the oldest and most frequently consumed alcoholic beverages to humankind is beer. Barley is the most commonly used grain for malting and beer making. But nowadays, various kinds of cereals and pseudo-cereals are used as alternative row materials: sorghum, rice, maize, millets, amaranth, buckwheat, quinoa, hulled wheats, teff, etc. [2–5]. In Africa, sorghum is used to produce various kinds of traditional beers named sorghum beers or opaque beers and known as *ikagage* in Rwanda [6], *pito* or *burukutu* in Nigeria and Ghana [7], *dolo* in Burkina Faso [8], *amgba* in Cameroon [6], *doro* or *chibuku* in Zimbabwe [9], *tchoukoutou* or *tchakpalo* in Togo and Benin [10,11] and *tchapalo* in Côte d'Ivoire [12].

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 [12,13]. It has a relatively low price compared to European beer, and therapeutic properties were assigned. It is considered a nutritious product because it contains a mixture of organic acids, alcohols, and other

growth factors produced by lactic acid bacteria and yeasts. Furthermore, it is rich in the B-group vitamins, including thiamine, folic acid, riboflavin, and nicotinic acid, and is high in essential amino acids such as lysine [14,15]. The beer is marketed and consumed while still actively fermenting and is effervescent and has a refreshing aroma. 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 fermentation), boiling the mixture, and pitching the boiled wort with dried yeast harvested from previous brews for alcoholic fermentation [16]. *Saccharomyces cerevisiae* was found as the predominant yeast species during alcoholic fermentation, while lactic acid fermentation is dominated by *Lactobacillus*; in particular, *Lb. fermentum*, *Lb. cellobiosus*, *Lb. brevis*, *Lb. coprophilus*, *Lb. plantarum*, and *Lb. hilgardii,* which is associated with *Enterococcus*, *Pediococcus*, and *Leuconostoc* [17,18]. The final beverage has a short shelf life and undergoes rapid deterioration within one to four days of production like most traditional African fermented foods. This results in heavy losses being incurred by the local brewers since the unsold batches have to be discarded. The deleterious changes are primarily due to the objectionable off-flavour or over-souring induced by continued microbial activities after production [19]. The short shelf life of *tchapalo* is one of the major deterrents to its large-scale production and development as a commercial product.

Due to the central role that sorghum beer has played in traditional society, it is important that the microbiology and biochemistry of the deterioration mechanisms are well understood. However, there is a limit to the number of studies carried out in this context, especially information available on the deterioration of *tchapalo*. The aim of this work was to evaluate the quality changes of *tchapalo* stored at different temperatures in order to define strategies for shelf life extension programs for this traditional beer.

2. Materials and Methods

2.1. Samples Collection and Storage Conditions

Samples of fresh *tchapalo* were collected from three traditional brewers, randomly identified at Williamsville-Macaci, Abobo-Habitat, and Cocody-Blockosso (Abidjan, Southern Côte d'Ivoire). Immediately after collection, the samples were cooled with ice and transferred to the laboratory within 2 h. Each sample was subdivided into volumes of 200 mL in sterile bottles and stored at ambient temperature (28 to 30 °C) or 4 °C in a refrigerator for four and six days, respectively. Every day, one bottle was taken out for analysis. All experiments were carried out independently in triplicate.

2.2. Enumeration of Microorganisms

The enumeration of microorganisms from the fresh and deteriorating *tchapalo* samples was carried out immediately on the following media: plate count agar (PCA; Conda, Madrid, Spain) for aerobic mesophilic bacteria (AM), Man Rogosa and Sharpe agar (MRS; Biokar Diagnoses, France) supplemented with cycloheximide for lactic acid bacteria (LAB), Sabouraud-chloramphenicol Agar (Biorad, France) for yeasts, and GYC agar (glucose 50 g/L; yeast extract 10 g/L; $CaCO_3$ 3 g/L; agar 15 g/L) supplemented with nystatin and penicillin for the isolation of acetic acid bacteria (AAB). The plates for LAB were incubated anaerobically for 48 h at 30 °C; those for AM and yeasts were incubated for 48 to 72 h at 30 °C, while the plates for AAB were incubated for five to seven days at 30 °C.

2.3. Physico-Chemical Analysis

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

2.4. Organic Acids Identification

Organic acids were analysed by high-performance liquid chromatography, following the method described by N'guessan et al. [20]. Briefly, the samples were firstly centrifuged at 3000 rpm for 20 min. Then, they were filtered through 0.45 µm Millipore membrane filters (Sartorius AG, Göttingen, Germany) and stored at −20 °C until analysis. 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). 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 eluent was 0.004 N H_2SO_4 with a flow rate of 0.8 mL/min, and the detector was set at 210 nm. Standard solutions (tannic, oxalic, citric, acetic, ascorbic, propionic, butyric, phtalic, salicylic, tartaric, malic, lactic, and fumaric acids) were purchased from Merck (Merck Co., Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich GmbH, Seelze, Germany) and were of analytical purity or for chromatographic use. They were prepared by the dilution of the individual compounds in distilled water.

2.5. Volatile Compounds Analysis

Volatile compounds were analyzed in a Shimadzu CG-14A gas chromatograph (Shimadzu Corporation). The instrument was equipped with a FID detector and a Porapak Q 100/120 column (1.80 m × 5 × 3). The fresh and deteriorating *tchapalo* samples (2 µL) were microfiltered through 0.2 µm Millipore membrane filters (Sartorius AG) and injected directly using the following temperature program: 2 min at 60 °C, increased to 150 °C at 10 °C/min, and held constant at 240 °C for 30 min. The detector temperature was set at 250 °C, and helium at 2 kg/cm^2 was used as the carrier gas. Standard solutions (ethanol, acetaldehyde, 1-propanol, 2-propanol, and 2-butanone; Merck, Darmstadt, Germany) were prepared by dilution of the individual compounds in distilled water.

2.6. Sensory Evaluation

A panel of 10 semi-trained judges, who are conventional/constant *tchapalo* consumers, was used to evaluate the beers to ascertain any difference between the control and the variant beers and also to assess their shelf lives. The judges were familiarized with the scoring scale and the sensory attributes to be evaluated during the preliminary training session. At each testing session, the judges were served a plate containing randomized three-digit coded cups with beer samples. A sample of a fresh brew of *tchapalo* was used as the reference beer. The samples were served at ambient temperature (28 to 30 °C) in the early morning in the sensory evaluation laboratory under normal lighting (standard white fluorescent lighting conditions). The judges were provided with water for mouth rinsing after each test. The panelists scored the samples for odor, appearance, and taste using a scoring scale with three categories [21,22] corresponding to 1 = fresh (samples similar to fresh *tchapalo* or without any off-odor), 2 = marginal (sample having slight difference or slight off-odor but still being acceptable), and 3 = spoiled (sample largely different from fresh *tchapalo* or producing strong off-odor). The time of sensory rejection was defined as the time when at least 50% of the panelists evaluated the samples to be in category 3.

2.7. Statistical Analysis

Analysis of variance (ANOVA) and Tukey HSD tests were performed with XLSTAT software 2014 (Addinsoft Inc., Brooklyn, NY, USA) to compare the variables analysed on the fresh and deteriorating samples. Statistical differences with $p < 0.05$ were considered significant. Principal component analysis (PCA) was used to compare *tchapalo* samples obtained from different storage conditions. PCA allowed the measured variables to be grouped into new variables called 'components' or 'factors.' This grouping is based on the correlation of the variables. The XLSTAT software (Addinsoft Inc.) was also used for the PCA.

3. Results

3.1. Evolution of Microbial Count

The evolution of AM, yeasts, LAB, and AAB concentration during *tchapalo* storage at different temperatures is shown in Figure 1. In the fresh *tchapalo*, the counts were 8.66 log CFU/mL, 8.05 log CFU/mL, 4.94 log CFU/mL, and 4.85 log CFU/mL for AM, Yeasts, LAB, and AAB, respectively. During storage at 28 to 30 °C (ambient temperature), AM slightly declined to reach a concentration of 8.57 log CFU/mL after four days. The yeast counts decreased the first two days to 7.88 log CFU/mL and then increased to 8.01 log CFU/mL after four days of storage. LAB were the lowest group of microorganism during storage. Like yeasts, the LAB counts firstly decreased and secondly increased to reach a concentration of 5.01 log CFU/mL. On the contrary, AAB grew throughout the storage time, reaching 6.52 log CFU/mL at the end of storage.

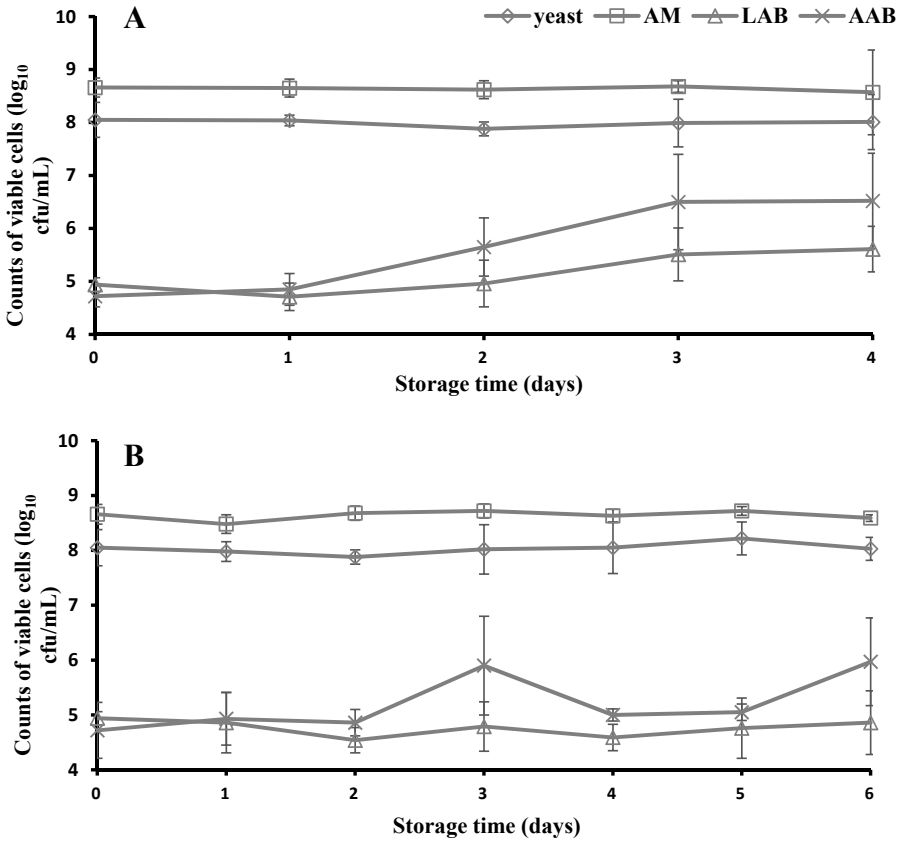

Figure 1. Evolution of aerobic mesophilic bacteria (AM), yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) of *tchapalo* stored at (**A**) ambient temperature (28 to 30 °C) for four days and (**B**) 4 °C for six days.

In samples stored at 4 °C, AM died off during the first day and then proliferated slowly and remained constant around 8.60 log CFU/mL until the end of storage. The yeast counts fluctuated during storage. They reached their maximal concentration of 8.22 log CFU/mL after five days of storage. LAB were also the lowest group of microorganism during storage at 4 °C. After the first two days of storage, they grew up to 4.86 log CFU/mL by the end of storage. During the first three days of storage, AAB counts increased, passing from 4.95 to 5.90 log CFU/mL. Afterward, AAB rapidly died off and then grew again to the level of 5.97 log CFU/mL after six days of storage.

3.2. Physico-Chemical Changes

Table 1 shows changes in pH, total titrable acidity (TTA), and total soluble solids (TSS) during *tchapalo* storage at ambient temperature (28 to 30 °C) and 4 °C. The initial values were 3.2, 0.9%, and 9.7°Brix for pH, TTA, and TSS, respectively. During storage at ambient temperature, the pH values slightly increased, passing from 3.2 to 3.4 after one day, and remained stable until the end of the storage period. The TTA values also remained stable throughout the storage period. On contrary, the TSS significantly decreased during the first day of storage, passing from 9.7 to 8°Brix. Thereafter, the TSS slightly decreased to reach 7.3°Brix after four days of storage.

At 4 °C, the pH values firstly increased and then decreased, but these variations were not significant. After six days of storage, the pH was 3.3. As expected, the TTA remained stable at 0.9% during the storage period. As at ambient temperature, the TSS values decreased during the storage period, but this decrease was not statistically significant. Thus, all the physico-chemical parameters globally remained stable during *tchapalo* storage at 4 °C.

Table 1. Evolution of the pH, total titratable acidity, and total soluble solids of *tchapalo* stored at (**A**) ambient temperature (28 to 30 °C) for four days and (**B**) 4 °C for six days.

A	**Storage Time (Days)**						
	0	**1**	**2**	**3**	**4**		
pH	3.2 ± 0.1 [a]	3.3 ± 0.1 [a]	3.4 ± 0.2 [a]	3.4 ± 0.2 [a]	3.4 ± 0.1 [a]		
Total titratable acidity (%)	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]		
Total soluble solids (°Brix)	9.7 ± 1.9 [a]	8.0 ± 1.6 [b]	7.8 ± 1.8 [b]	7.5 ± 1.7 [b]	7.3 ± 1.8 [b]		
B	**Storage time (Days)**						
	0	**1**	**2**	**3**	**4**	**5**	**6**
pH	3.2 ± 0.1 [a]	3.4 ± 0.1 [a]	3.4 ± 0.1 [a]	3.4 ± 0.1 [a]	3.3 ± 0.1 [a]	3.3 ± 0.2 [a]	3.3 ± 0.2 [a]
Total titratable acidity (%)	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]	0.9 ± 0.1 [a]
Total soluble solids (°Brix)	9.7 ± 1.9 [a]	9.6 ± 1.8 [a]	9.5 ± 2.1 [a]	9.6 ± 2.1 [a]	9.5 ± 2.2 [a]	9.4 ± 2.1 [a]	9.3 ± 2.1 [a]

The values are the means of nine independent trials with *tchapalo* samples from three local producers' ± standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$).

3.3. Organics Acid Contents of the Beers

As shown in Table 2, only six organic acids were regularly found in *tchapalo* during storage at ambient temperature and 4 °C out of the thirteen compounds analyzed. These acids were tannic, oxalic, tartaric, lactic, citric, and acetic acids, among which lactic acid, followed by tartaric acid, were the major compounds. The others were not found or were in traces. During storage at ambient temperature, the lactic acid content slightly increased, passing from 72.76 to 79.11 mg/mL, then decreased to reach 54.94 mg/mL at the end of the storage period. The evolution of tannic, oxalic and tartaric acids followed a similar trend. They slightly decreased throughout the storage period, so they passed from 0.20 to 0.18 mg/mL, from 0.26 to 0.20 mg/mL, and from 1.83 to 0.81 mg/mL, respectively, for tannic, oxalic, and tartaric acids. Citric acid, which was not detected in fresh *tchapalo*, was found at 0.31 mg/mL after one day of storage and remained stable until the end of the storage period. Acetic acid, on the contrary, was detected only at three days of storage at a concentration of 1.26 mg/mL. The total organic acid contents showed fluctuation during the storage period. The highest value (81.21 mg/mL) was reached after one day of storage and the lowest value (56.45 mg/mL) after four days.

In *tchapalo* stored at 4 °C, the lactic acid contents remained globally stable throughout the storage period. The values were between 66.60 and 83.57 mg/mL. The tannic, oxalic, and tartaric acids also remained stable, and acetic acid was not detected. The total organic acid contents showed fluctuations, with the highest value (85.40 mg/mL) at six days of storage and the lowest value (68.44 mg/mL) at two days.

Table 2. Organic acids detected during the storage of *tchapalo* at (**A**) ambient temperature (28 to 30 °C) for four days and (**B**) 4 °C for six days.

A	**Storage Time (Days)**						
Compound Detected (mg/mL)	**0**	**1**	**2**	**3**	**4**		
Tannic acid	0.20 ± 0.11 ^{a}	0.22 ± 0.15 ^{a}	0.20 ± 0.11 ^{a}	0.19 ± 0.13 ^{a}	0.18 ± 0.08 ^{a}		
Oxalic acid	0.26 ± 0.04 ^{a}	0.23 ± 0.06 ^{a}	0.24 ± 0.09 ^{a}	0.23 ± 0.15 ^{a}	0.20 ± 0.05 ^{a}		
Tartaric acid	1.83 ± 0.18 ^{a}	1.34 ± 0.45 ^{a}	0.97 ± 0.23 ^{a}	0.77 ± 0.42 ^{a}	0.81 ± 0.13 ^{a}		
Lactic acid	72.76 ± 0.02 ^{a}	79.11 ± 2.08 ^{a}	64.42 ± 2.24 ^{ab}	57.35 ± 2.44 ^{b}	54.94 ± 1.86 ^{b}		
Citric acid	<0.001	0.31 ± 0.06 ^{b}	0.37 ± 0.05 ^{b}	0.31 ± 0.07 ^{b}	0.32 ± 0.08 ^{b}		
Acetic acid	<0.001	<0.001	<0.001	1.26 ± 0.01	<0.001		
Total	75.05	81.21	66.2	78.21	56.45		
B	**Storage time (Days)**						
Compound detected (mg/mL)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Tannic acid	0.20 ± 0.11 ^{a}	0.21 ± 0.15 ^{a}	0.16 ± 0.08 ^{a}	0.21 ± 0.13 ^{a}	0.20 ± 0.11 ^{a}	0.18 ± 0.09 ^{a}	0.20 ± 0.11 ^{a}
Oxalic acid	0.26 ± 0.04 ^{a}	0.24 ± 0.09 ^{a}	0.20 ± 0.09 ^{a}	0.24 ± 0.05 ^{a}	0.24 ± 0.11 ^{a}	0.25 ± 0.04 ^{a}	0.27 ± 0.12 ^{a}
Tartaric acid	1.83 ± 0.18 ^{a}	1.74 ± 0.79 ^{a}	1.48 ± 0.60 ^{a}	1.76 ± 0.40 ^{a}	1.71 ± 0.84 ^{a}	1.59 ± 0.41 ^{a}	1.36 ± 0.68 ^{a}
Lactic acid	72.76 ± 1.90 ^{a}	68.83 ± 2.12 ^{a}	66.60 ± 3.20 ^{a}	74.77 ± 1.17 ^{a}	66.87 ± 1.17 ^{a}	70.08 ± 2.29 ^{a}	83.57 ± 3.17 ^{a}
Total	75.05	71.02	68.44	76.98	69.04	72.1	85.4

The values are the means of nine independent trials with *tchapalo* samples from three local producers' ± standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$).

3.4. Evolution of Volatile Compounds

Ethanol was the only volatile compound regularly found in this study (Table 3). During storage at ambient temperature, its content increased significantly from 4.57% (fresh *tchapalo*) to 5.62% during the first two days. Subsequently, it decreased to 4.86% at the end of the storage time. On the contrary, in *tchapalo* stored at 4 °C, the ethanol contents were statistically identical throughout the storage period. The values were between 3.63% and 4.57% and were lower than those for ambient temperature.

Table 3. The evolution of volatile compounds detected in *tchapalo* stored at (**A**) ambient temperature (28 to 30 °C) for four days and (**B**) 4 °C for six days.

A	**Storage Time (Days)**						
Concentration (%)	**0**	**1**	**2**	**3**	**4**		
Ethanol	4.57 ± 0.84 ^{a}	5.04 ± 1.57 ^{a}	5.62 ± 2.23 ^{b}	5.1 ± 2.49 ^{a}	4.86 ± 1.21 ^{a}		
2-Butanone	nd	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	nd		
1-Propanol	nd	nd	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}		
B	**Storage time (Days)**						
Concentration (%)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Ethanol	4.57 ± 0.84 ^{a}	3.86 ± 1.28 ^{a}	4.36 ± 1.42 ^{a}	4.15 ± 1.3 ^{a}	3.63 ± 0.56 ^{a}	4.21 ± 0.97 ^{a}	3.91 ± 0.23 ^{a}
2-Butanone	nd	0.02 ± 0.01 ^{a}	nd	0.02 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}	0.02 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}
1-Propanol	nd	0.02 ± 0.00	nd	nd	nd	nd	nd

The values are the means of nine independent trials with *tchapalo* samples from three local producers' ± standard deviations. On the same line, mean values with the same letter are not significantly different ($p > 0.05$); nd = not detected.

3.5. Sensory Quality of the Beers

The results of the sensory evaluation of *tchapalo* stored at ambient temperature and at 4 °C are presented in Table 4. After one day of storage at ambient temperature, 50% (mean percentage of nine independent trials, each assessed by 10 panelists) of the panelists noticed that the taste of the beers was marginal, while 38.9% of them judged the taste as spoiled and 11.1% as fresh. After two days, 75.1% of panelists reported that the taste was spoiled. As with taste, more than half of the panelists (52.4%) judged the odor as marginal after one day of storage, 34% judged it as spoiled, and 13.6% judged it as fresh. However, after two days, 69.2% of the panelists recorded the odor as spoiled. The appearance

of the beer was also judged as marginal after one day of storage by 61.8% of panelists and as spoiled by 20.6%. After two days, 64.7% of panelists judged that the appearance of the beer was spoiled. Thus, the sensory rejection time, defined as the time when at least 50% of the panelists evaluated the analyzed attributes of *tchapalo* as spoiled, was two days after storage at ambient temperature (28 to 30 °C). At 4 °C, the rejection time was three days. Taste was the first attribute to be spoiled, according to the panelists, followed by odor and appearance.

Table 4. A sensory analysis of *tchapalo* stored at (**A**) ambient temperature (28 to 30 °C) for four days and (**B**) 4 °C for six days.

A		**Storage Time (Days)**					
Attributes		**1**	**2**	**3**	**4**		
Appearance	Fresh	18	6.8	2.2	1.1		
	Marginal	61.8	28.5	11.4	11.1		
	Spoiled	20.6	64.7	86.4	97.8		
Odor	Fresh	13.6	6.8	3.3	1.1		
	Marginal	52.4	24	12.5	3.3		
	Spoiled	34	69.2	84.2	95.6		
Taste	Fresh	11.1	2.2	1.1	0		
	Marginal	50	22.7	11.3	1.1		
	Spoiled	38.9	75.1	87.6	98.9		
B		**Storage time (Days)**					
Attributes		**1**	**2**	**3**	**4**	**5**	**6**
Appearance	Fresh	28.8	13.6	2.2	2.2	3.3	0
	Marginal	45.5	40.1	22.5	14.7	7.8	0
	Spoiled	25.7	46.3	75.3	83.1	88.9	100
Odor	Fresh	28.6	13.7	4.5	1.1	4.4	0
	Marginal	44.7	44.5	20.2	14.3	5.5	0
	Spoiled	26.7	41.8	75.3	84.6	90.1	100
Taste	Fresh	19.2	11.3	5.6	4.5	2.2	0
	Marginal	52.8	34.1	19.2	9	2.2	0
	Spoiled	28	54.6	75.2	86.5	95.6	100

3.6. Principal Components of Tchapalo during Storage

In an attempt to simplify the interpretation of the data, principal component analysis was applied to the biochemical data. As shown in Table 5, the ten measured variables of *tchapalo* stored at different temperatures were reduced to two main components (F1 and F2) by the PCA. F1 and F2 explained 87.88% and 60.20% of the total data variance, respectively, for *tchapalo* stored at ambient temperature and 4 °C. The PCA showed that, at ambient temperature, the variables that mainly contributed positively (F loadings > 0.8) to F1 were pH, citric acid, and 1-proponal, and those that contributed positively to F2 were ethanol and 2-butanone. The TSS and the lactic and tartaric acids contributed negatively to F1. With data obtained at 4 °C, F1 essentially describes 1-propanol on the positive side, while F2 describes 2-butanone and TSS on the positive and negative sides, respectively.

When stored beers were plotted in the space created by the two dimensions, the results showed that beers at ambient temperature were widely separated from the reference one (Figure 2A). Thus, fresh *tchapalo* was found to be on the negative side of F1 while beer stored for three days (Day 3) was on the positive side of F1. On the contrary, beers stored at 4 °C were more or less close to each other in terms of results (Figure 2B).

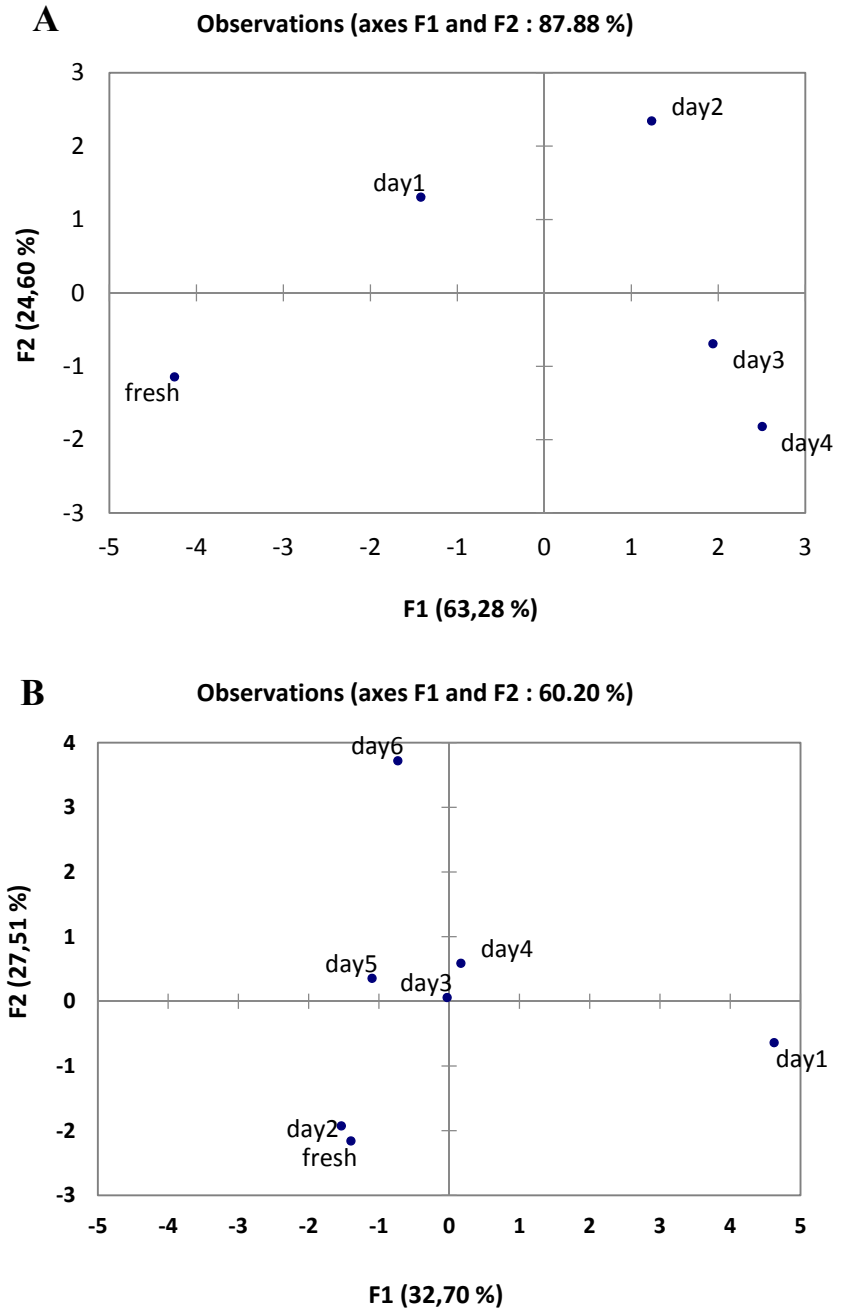

Figure 2. Plot of the two principal components of the principal component analysis (PCA) of *tchapalo* stored at (**A**) ambient temperature (28 to 30 °C) for four days and (**B**) 4 °C for six days.

Table 5. The principal component loadings resulting from the principal component analysis of *tchapalo* stored at (**A**) ambient temperature (28 to 30 °C) for four days and (**B**) 4 °C for six days.

A	**F1**	**F2**
pH	0.9956	0.0670
Total soluble solids	−0.9342	−0.2012
Ethanol	0.5491	0.8083
Tannic acid	−0.5865	0.6671
Oxalic acid	−0.7804	0.2879
Lactic acid	−0.8165	0.4439
Tartaric acid	−0.9938	−0.0224
Citric acid	0.8480	0.4574
1-Propanol	0.8699	−0.4126
2-Butanone	0.2824	0.8104
B	**F1**	**F2**
pH	0.4939	0.0828
Total soluble solids	0.0593	−0.8778
Ethanol	−0.5380	−0.6067
Tannic acid	0.6213	0.1954
Oxalic acid	−0.0035	0.5693
Lactic acid	−0.1932	0.7198
Tartaric acid	0.3361	−0.6014
Fumaric acid	0.0155	0.2402
1-Propanol	0.9532	−0.1439
2-Butanone	0.3528	0.8907

4. Discussion

The present study aimed to evaluate the quality change of *tchapalo*, an Ivoirian traditional sorghum beer, during storage at different temperatures. The initial total aerobic counts obtained in this study (8.66 log CFU/mL) are lower than those reported by Sanni et al. [23] for *burukutu* and *pito*, the sister beers of *tchapalo* from Nigeria. Yeasts, with an initial count of 8.05 log CFU/mL, are the dominant microorganisms in *tchapalo*, followed by LAB, as reported by Aka et al. [13]. The dominance of yeast counts is due to the fact that brewers use dried yeast harvested from previous brews to inoculate the sorghum wort [12]. By this practice, the initial phase of the alcoholic fermentation process is shortened, the risk of fermentation failure is reduced, and it results in the promotion of desirable changes during the fermentation process [14]. LAB, on contrary, come from the environment and conduce lactic fermentation [24]. A symbiotic relationship could explain the simultaneous presence of yeasts and LAB. The latter create an acidic environment favorable to the proliferation of yeasts, while yeasts produce vitamins and increase other factors such as amino acids to aid the growth of LAB [25].

During the storage period, the aerobic mesophilic bacteria and yeast counts remained stable. These findings disagree with the report of Sanni et al. [23] on sorghum beers produced in Nigeria. They found significant differences in the viable counts of microorganisms in deteriorating beverage samples and stated that the reduction in the microbial population indicated the gradual dying off of some initial microflora in the fermentation. Here, the stable count of yeasts could be related to the fact that only a few species were present in the yeast population. Indeed, several authors showed that *Saccharomyces cerevisiae* represent up to 99% of yeast species in sorghum beers [26,27]. N'guessan et al. [18] found *S. cerevisiae* at a frequency of 87.36%, followed by *Candida tropicalis* (5.45%) and *Meyerozyma caribbica* (2.75%), in *tchapalo* samples. *Candida* species were thought to produce fruity odors and pellicle during spoilage [28].

In modern beers, LAB can be found at almost every stage of the malting and brewing process and are recognized as the most hazardous bacteria for breweries, being responsible for approximately 70% of microbial beer-spoilage incidents, which negatively affect the product quality and produce important economic losses for the brewing industry [29–32]. However, during *tchapalo* production, they

are very important as they conduce lactic fermentation. In this study, LAB counts, in contrast to yeasts, decreased first and then increased significantly according to the storage temperature. The variation in the LAB population may be influenced by factors such as the nutrients available for growth, i.e., carbon and nitrogen sources, the pH, the oxygen level, and the type of microorganisms present in the samples during succession. According to Lyumugabe et al. [25], the metabolic activities of mesophilic lactic acid bacteria are primarily responsible for spoilage. These bacteria, along with other undesirable bacteria (*Acetobacter*), produce acetic acid, volatile off-flavors, fruity odors, and pellicles, which render the taste, odor, and texture of the beer unacceptable to consumers. *Lactobacillus brevis*, *Pediococcus damnosus*, and *Pediococcus claussenii* represent the most important beer-spoilage bacteria [33]. Despite these detrimental metabolic activities, some LAB may play a positive role in the brewing process by eliminating undesirable microorganisms such as beer-spoilage LAB and by contributing to wort bioacidification [32,34]. LAB may account for food biological preservation mainly by the production of ribosomally-synthesized antimicrobial peptides, referred to as bacteriocins [35–37].

The microbial counts of AAB increased gradually until the end of the storage period at ambient temperature and peaked at Day 3 when the samples were stored at 4 °C. This rise in the AAB counts could be a result of the conversion of ethanol to acetic acid in the presence of a small concentration of oxygen in the deteriorating *tchapalo*. The overoxidation of ethanol could explain its low concentration during storage. In fact, AAB can metabolize a variety of organic acids such as acetic, citric, fumaric, lactic, malic, pyruvic, and succinic acids. It was reported that *Acetobacter* species oxidized ethanol into acetic acid and, subsequently, completed the oxidation of acetic acid into water and CO_2 [38]. AAB have been also reported in deteriorating *pito* and *burukutu* in concentrations of about 10^2 to 10^4 CFU/mL [23], which were lower than those found in this study. The role of AAB during fermentation is related to acetic acid production, which comprises part of the aroma volatiles. However, AAB can be considered spoilage microorganisms, when the sample becomes unacceptable to consumers. In addition, like LAB, AAB also can contribute to the acidification and inhibition of undesirable microorganisms [39].

Despite the significant growth in LAB and AAB counts during the storage period, the pH and TTA remained stable. Aka et al. [13] and Sanni et al. [23] found similar results during alcoholic fermentation in *tchapalo* production and during *pito* storage at ambient temperature, respectively. This could mean that acids produced by these bacteria were not sufficient to raise the pH significantly in the deteriorating beverages. Thus acidity could not be used as an indicator of *tchapalo* spoilage. The decrease in TSS contents when the experiment was conducted at ambient temperature meant the fast utilization of available solids. Not all dissolved solids were available for utilization by the microorganisms present, as shown by the levelling off of the solids in the products at the end of the storage period. The levelling off may be due to the inhibition effects of ethanol. The toxicity of ethanol to bacteria and yeasts physiology is diverse, though cellular membranes appear to be the main sites of ethanol damage. The specific effects include growth inhibition, reduced cell size [40], reduced respiration and glucose uptake [41], enzyme inactivation, lipid modification, a loss of proton motive force across the plasma membrane [42], and an increase in membrane permeability [43]. In this study, the ethanol contents increased significantly during the first two days of storage at ambient temperature, so ethanol could act as the major factor in controlling the utilization of the available solids. The subsequent decrease in the ethanol content of the deteriorating beverages is attributable to the activities of the AAB, which oxidise the ethanol to acetic acid. It should be mentioned here that strict anaerobic conditions are never achieved during the traditional brewing of *tchapalo*, thus creating an enabling environment for the acetic acid bacteria to become established and initiate spoilage.

The presence of weak organic acids mainly determines the acidity, which is one of the most important organoleptic parameters in *tchapalo*. As mentioned by Herrero et al. [44], for wine, the composition and concentration of each acid is essential for the quality of the final product. They also act as a buffer; thus the cytosolic pH of yeast and its metabolism during fermentation cannot be affected [45]. Most organic acids present in *tchapalo* were not consumed or produced

sufficiently during storage. Meanwhile, we observed slight fluctuations. According to Paraggio and Fiore [46], acetic acid is the main volatile acid in fermented beverages, and it is recognized as one of the by-products that have the most negative effects on the analytical profile of alcoholic beverages. Thus some authors mentioned that it must be absent in beers [23,47]. We found this acid at a detectable level only after the third day of storage at ambient temperature, contrary to Sanni et al. [23], who detected it in the fresh and deteriorating samples of *pito* and *burukutu*.

Although most of the parameters analyzed in this study did not vary significantly, the panelists rejected the samples at Day 2 and day 3 for *tchapalo* stored at ambient temperature and 4 °C, respectively. This observation may indicate that there are other compounds that mainly determine the acceptability of *tchapalo* for consumers. These compounds could be used as indicators of the spoilage or acceptability of the product. The rejection times found here are not totally unexpected since the literature mentioned that African beverages have short shelf lives and undergo rapid deterioration within 48 h of production [23,25].

5. Conclusions

In conclusion, the present work shows that *tchapalo* is spoiled after two days at ambient temperature and after three days at a refrigerated temperature (4 °C), but the biochemical compounds analyzed in this study were not sufficient to characterize the spoiled *tchapalo* samples. Only slight fluctuations were observed for most of them. As the production of this beverage has important implications for the food system and economy of the country, further studies are required in order to find the compounds which mainly determine the acceptability or rejection of the product. So, a better preservation strategy could be proposed.

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References

1. Dietler, M. Alcohol: Anthropological/archaeological perspectives. *Annu. Rev. Anthropol.* **2006**, *35*, 229–249. [CrossRef]
2. De Meo, B.; Freeman, G.; Marconi, O.; Booer, C.; Perretti, G.; Fantozzi, P. Behaviour of malted cereals and pseudo-cereals for gluten-free beer production. *J. Inst. Brew.* **2011**, *117*, 541–546.
3. Mayer, H.; Marconi, O.; Perretti, G.; Sensidoni, M.; Fantozzi, P. Investigation on suitability of hulled wheats for malting and brewing. *J. Am. Soc. Brew. Chem.* **2011**, *69*, 116–120.
4. Di Ghionno, L.; Sileoni, V.; Marconi, O.; De Francesco, G.; Perretti, G. Comparative study on quality attributes of gluten-free beer from malted and unmalted teff [Eragrostis tef (zucc.) trotter]. *LWT - Food Sci. Technol.* **2017**, *84*, 746–752. [CrossRef]
5. Marconi, O.; Sileoni, V.; Ceccaroni, D.; Perretti, G. The Use of Rice in Brewing. In *Rice*; Li, J., Ed.; Open Access Book in Press; ISBN 978-953-51-4979-8-2. 2016; Available online: https://www.intechopen.com/books/advances-in-international-rice-research/the-use-of-rice-in-brewing (accessed on 28 August 2017). [CrossRef]
6. Lyumugabe, L.; Kamaliza, G.; Bajyana, E.; Thonart, P.H. Microbiological and physico-chemical characteristics of Rwandese traditional beer "Ikigage". *Afr. J. Biotechnol.* **2010**, *9*, 4241–4246.
7. Achi, O.K. The potential for upgrading traditional fermented foods through biotechnology. *Afr. J. Biotechnol.* **2005**, *5*, 375–380.
8. Dicko, M.H.; Gruppen, H.; Traore, A.S.; Voragen, A.G.J.; Van Berkel, W.J.H. Sorghum grain as human food in Africa: Relevance of content of starch and amylase activities. *Afr. J. Biotechnol.* **2006**, *5*, 384–395.
9. Chamunorwa, A.T.; Feresu, S.B.; Mutukumira, A.N. Identification of lactic acid bacteria isolated from opaque beer (Chibuku) for potential use as a starter culture. *J. Food Technol. Afr.* **2002**, *7*, 93–97.
10. Kayodé, A.P.P.; Adegbidi, A.; Hounhouigan, J.D.; Linnemann, A.R.; Nout, M.J.R. Quality of farmer's varieties of sorghum and derived foods as perceived by consumers in Benin. *Ecol. Food Nutr.* **2005**, *44*, 271–294. [CrossRef]

11. Osseyi, E.G.; Tagba, P.; Karou, S.D.; Ketevi, A.P.; Lamboni, C.R. Stabilization of the traditional sorghum beer, "tchoukoutou" using rustic wine-making method. *Adv. J. Food Sci. Technol.* **2011**, *3*, 254–258.
12. Djè, K.M.; N'guessan, K.F.; Djéni, N.T.; Dadié, A.T. Biochemical changes during alcoholic fermentation in the production of ''tchapalo'', a traditional sorghum beer. *Int. J. Food Eng.* **2008**, *4*. [CrossRef]
13. Aka, S.; Djéni, T.; N'guessan, F.; Yao, C.; Djè, M. Variabilité des propriétés physico-chimiques et dénombrement de la flore fermentaire du tchapalo, une bière traditionnelle de sorgho en Côte d'ivoire. *Afr. Sci.* **2008**, *4*, 274–286. [CrossRef]
14. Holzapfel, W. Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int. J. Food Microbiol.* **2002**, *75*, 197–212. [CrossRef]
15. Kayodé, A.P.P.; Hounhouigana, J.D.; Nout, M.J.R.; Niehof, A. Household production of sorghum beer in Benin: Technological and socioeconomic aspects. *J. Food Technol. Afr.* **2007**, *31*, 258–264. [CrossRef]
16. Aka, S.; Camara, F.; Nanga, Y.Z.; Loukou, Y.G.; Dje, K.M. Evaluation of organic acids and sugars contents during the production of tchapalo, a traditional sorghum beer of Côte d'Ivoire. *J. Food Technol.* **2008**, *6*, 189–195.
17. Djè, K.M.; Aka, S.; Zinzendorf, N.Y.; Yao, K.C.; Loukou, Y.G. Predominant lactic acid bacteria involved in the spontaneous fermentation step of tchapalo process, a traditional sorghum beer of Côte d'Ivoire. *Res. J. Biol. Sci.* **2009**, *4*, 789–795.
18. N'guessan, K.F.; Brou, K.; Jacques, N.; Casaregola, S.; Djè, K.M. Identification of yeasts during alcoholic fermentation of tchapalo, a traditional sorghum beer from Côte d'Ivoire. *Antonie Van Leeuwenhoek* **2011**, *99*, 855–864. [CrossRef] [PubMed]
19. Kutyauripo, J.; Parawira, W.; Tinofa, S.; Kudita, I.; Ndengu, C. Investigation of shelf-life extension of sorghum beer (Chibuku) by removing the second conversion of malt. *Int. J. Food Microbiol.* **2009**, *129*, 271–276. [CrossRef] [PubMed]
20. N'guessan, F.K.; N'dri, D.Y.; Camara, F.; Djè, M.K. *Saccharomyces cerevisiae* and Candida tropicalis as starter cultures for the alcoholic fermentation of tchapalo, a traditional sorghum beer. *World J. Microbiol. Biotechnol.* **2010**, *26*, 693–699.
21. Mejlholm, O.; Kjeldgaard, J.; Modberg, A.; Vest, M.B.; Bøknæs, N.; Koort, J.; Bjorkroth, J.; Dalgaard, P. Microbial changes and growth of Listeria monocytogenes during chilled storage of brined shrimp (Pandalus borealis). *Int. J. Food Microbiol.* **2008**, *124*, 250–259. [CrossRef]
22. Argyri, A.A.; Panagou, E.Z.; Tarantilis, P.A.; Polysiou, M.; Nychas, G.J.E. Rapid qualitative and quantitative detection of beef fillets spoilage based on Fourier transform infrared spectroscopy data and artificial neural networks. *Sens. Actuators B Chem.* **2010**, *145*, 146–154. [CrossRef]
23. Sanni, A.I.; Onilude, A.A.; Fadahunsi, I.F.; Afolabi, R.O. Microbial deterioration of traditional alcoholic beverages in Nigeria. *Food Res. Int.* **1999**, *32*, 163–167. [CrossRef]
24. Aka, S.; N'guessan, K.F.; Nanga, Y.Z.; Loukou, Y.G.; Mazabraud, A.I.; Djè, K.M. Characterization of Lactobacillus species isolated from mash, sour wort and tchapalo produced in Côte d'Ivoire. *Food* **2010**, *4*, 49–54.
25. Lyumugabe, F.; Gros, J.; Nzungize, J.; Bajyana, E.; Thonart, P. Characteristics of African traditional beers brewed with sorghum malt: A review. *Biotechnol. Agron. Soc. Environ.* **2012**, *16*, 509–530.
26. Van der Aa Kühle, A.; Jespersen, L.; Glover, R.L.K.; Diawara, B.; Jakobsen, M. Identification and characterization of *Saccharomyces cerevisiae* strains isolated from West African. *Yeast* **2001**, *18*, 1069–1079. [CrossRef]
27. Glover, R.L.K.; Abaidoo, R.C.; Jakobsen, M.; Jespersen, L. Biodiversity of *Saccharomyces cerevisiae* isolated from a survey of pito production sites in various parts of Ghana. *Syst. Appl. Microbiol.* **2005**, *28*, 755–761. [CrossRef]
28. Haggblade, S.; Holzapfel, H. Industrialization of Africa's indigenous beer brewing. In *Industrialization of Indigenous Fermented Foods*; Streinrous, K.H., Ed.; CRC Press: New York, NY, USA, 2004.
29. Jespersen, L.; Jakobsen, M. Specific spoilage organisms in breweries and laboratory media for their detection. *Int. J. Food Microbiol.* **1996**, *33*, 139–155. [CrossRef]
30. Sakamoto, K.; Konings, W.N. Beer spoilage bacteria and hop resistance. *Int. J. Food Microbiol.* **2003**, *89*, 105–124. [CrossRef]

31. March, C.; Manclús, J.J.; Abad, A.; Navarro, A.; Montoya, A. Rapid detection and counting of viable beer-spoilage lactic acid bacteria using a monoclonal chemiluminescence enzyme immunoassay and a CCD camera. *J. Immunol. Methods* **2005**, *303*, 92–104. [CrossRef] [PubMed]
32. Vaughan, A.; O'Sullivan, T.; van Sinderen, D. Enhancing the microbiological stability of malt and beer—A review. *J. Inst. Brew.* **2005**, *111*, 355–371. [CrossRef]
33. Holzapfel, W.; Franz, C.M.A.P.; Ludwig, W.; Back, W.; Dicks, L.M.T.D. The genera Pediococcus and Tetragenococcus. In *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*; Dworkin, M., Ed.; Springer: New York, NY, USA, 2005.
34. Lewis, D. Biological mash and wort acidification. *New Brewer* **1998**, *15*, 36–45.
35. Cintas, L.M.; Casaus, P.; Herranz, C.; Nes, I.F.; Hernández, P.E. Bacteriocins of lactic acid bacteria. *Food Sci. Technol. Int.* **2001**, *7*, 281–305. [CrossRef]
36. Cleveland, J.; Montville, T.J.; Nes, I.F.; Ckikindas, M.L. Bacteriocins: Safe, natural antimicrobials for food preservation. *Int. J. Food Microbiol.* **2001**, *71*, 1–20. [CrossRef]
37. Cotter, P.D.; Hill, C.; Ross, R.P. Bacteriocins: Developing innate immunity for food. *Nat. Rev. Microbiol.* **2005**, *3*, 777–788. [CrossRef] [PubMed]
38. Sengun, I.Y.; Karabiyikli, S. Importance of acetic acid bacteria in food industry. *Food Control.* **2011**, *22*, 647–656. [CrossRef]
39. Ouoba, L.I.; Kando, C.; Parkouda, C.; Sawadogo-Lingani, H.; Diawara, B.; Sutherland, J.P. The microbiology of Bandji, palm wine of Borassusakeassii from Burkina Faso: Identification and genotypic diversity of yeasts, lactic acid and acetic acid bacteria. *J. Appl. Microbiol.* **2012**, *113*, 1428–1441. [CrossRef] [PubMed]
40. Canetta, E.; Adya, A.K.; Walker, G.M. Atomic force microscopic study of the effects of ethanol on yeast cell surface morphology. *FEMS Microbiol. Lett.* **2006**, *255*, 308–319. [CrossRef] [PubMed]
41. Pascual, C.; Alonso, A.; Garcia, I.; Romay, C.; Kotyk, A. Effect of ethanol on glucose transport, key glycolytic enzymes, and proton extrusion in *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* **1988**, *32*, 374–378. [CrossRef] [PubMed]
42. Mizoguchi, H.; Hara, S. Ethanol-induced alterations in lipid composition of *Saccharomyces cerevisiae* in the presence of exogenous fatty acid. *J. Ferment. Bioeng.* **1997**, *83*, 12–16. [CrossRef]
43. Marza, E.; Camougrand, N.; Manon, S. Bax expression protects yeast plasma membrane against ethanol-induced permeabilization. *FEBS Lett.* **2002**, *521*, 47–52. [CrossRef]
44. Herrero, M.; Cuesta, I.; Garcia, L.A.; Diaz, M. Change in organic acids during malolactic fermentation at different temperatures in yeast fermented apple juice. *J. Inst. Brew.* **1999**, *105*, 191–195. [CrossRef]
45. Torija, M.J.; Beltran, G.; Novo, M.; Poblet, M.; Guillamon, J.M.; Mas, A.; Rozes, N. Effects of fermentation temperature and Saccharomyces species on the cell fatty acid composition and presence of volatile compounds in wine. *Int. J. Food Microbiol.* **2003**, *85*, 127–136. [CrossRef]
46. Paraggio, M.; Fiore, C. Screening of *Saccharomyces cerevisiae* wine strains for the production of acetic acid. *World J. Microbiol. Biotechnol.* **2004**, *20*, 743–747. [CrossRef]
47. Odunfa, S.A. African fermented foods. In *Microbiology of Fermented Foods*; Wood, B.J.B., Ed.; Elsevier Applied Science Publishers: New York, NY, USA, 1985.