



# Preliminary Studies on the Variation in Microbial Succession, Physico-Chemical Characteristics and Antioxidant Capacity during a Spontaneous Fermentation of *Mutchayan*, a Traditional Fermented Baobab Derived Food <sup>†</sup>

Ahotondji Mechak Gbaguidi <sup>1,2</sup> , Flora Josiane Chadare <sup>1,2,\*</sup>, Sègla Wilfrid Padonou <sup>1,2</sup> ,  
Comlanvi Oscar Assou <sup>2</sup> and Djidjoho Joseph Hounhouigan <sup>2</sup>

<sup>1</sup> Laboratoire de Sciences et Technologie des Aliments et Bioressources et de Nutrition Humaine, Université Nationale d'Agriculture (LaSTAB-NH/UNA), Centre Universitaire de Sakété, Sakété BP 114, Benin

<sup>2</sup> Laboratoire de Sciences et Technologie des Aliments, Ecole de Nutrition et des Sciences et Technologies des Aliments, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi (LaSTA/ENSTA/FSA/UAC), Jéricho 03 BP 2819, Benin

\* Correspondence: fchadare@gmail.com

<sup>†</sup> Presented at the 2nd International Electronic Conference on Foods—Future Foods and Food Technologies for a Sustainable World, 15–30 October 2021; Available online: <https://foods2021.sciforum.net/>.

**Abstract:** *Mutchayan* is a traditionally fermented cooked cereal dough mixed with baobab pulp, consumed in Benin. The study evaluated the physico-chemical and microbiological changes of *Mutchayan* during 0 to 120 h of spontaneous fermentation. An analysis of the studied fermentation process revealed an increase in titratable acidity and antioxidant capacity (between 0 and 120 h) and a decrease in ascorbic acid content during the first 24 h of the fermentation. Dry matter content and Brix value decreased from 18.7 to 16.7 g/100 g and 7.6 to 5.0 °Brix, respectively, while pH did not change notably. Microbiological analysis revealed the presence of molds at the beginning of the fermentation, which was inhibited after 36 h, while the lactic acid bacteria and yeasts dominated the process.

**Keywords:** presumptive lactic acid bacteria; fermentation; yeast; antioxidant capacity; ascorbic acid



**Citation:** Gbaguidi, A.M.; Chadare, F.J.; Padonou, S.W.; Assou, C.O.; Hounhouigan, D.J. Preliminary Studies on the Variation in Microbial Succession, Physico-Chemical Characteristics and Antioxidant Capacity during a Spontaneous Fermentation of *Mutchayan*, a Traditional Fermented Baobab Derived Food. *Biol. Life Sci. Forum* **2021**, *6*, 124. <https://doi.org/10.3390/Foods2021-10989>

Academic Editor: Theodoros Varzakas

Published: 21 December 2022



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Fermentation is an old technique for food preservation, widely used [1]. It is a process based on the biological activity of microorganisms for increasing food value through the development of bioactive compounds, and bacteriocins able to limit the growth of undesirable microorganisms in foods. It involves different categories of microorganisms depending on the product and the type of fermentation [2]: acid fermentation, alcoholic fermentation, or alkaline fermentation. Materials rich in starch or glucose such as cereals, tubers, and roots, are usually submitted to acid and alcoholic fermentations. Acid fermentation improves the product flavor, the content of the bioactive compounds, and the bioavailability of the minerals [3]. It delays starch bioavailability [3] and provides probiotics for human health [4].

The acid fermentation of cereals has contributed to several products such as *Maivè* [5], *Ogi* [6] derived from maize, *Gowé* [7] derived from sorghum, and *Mutchayan* [8] derived from cereals (maize or sorghum or millet) and baobab fruit pulp. *Mutchayan* is a traditional food of the “Otamari” socio-cultural group of northern Benin. It is derived from spontaneous fermentation in a jar, of a cooked dough produced with cereal flour (maize/millet/sorghum) and baobab (*Adansonia digitata*) fruit pulp; the fermentation can last 24 to 168 h. The fermented product is consumed by rural populations, as a tonifying drink, after its dilution [8]. *Mutchayan* has a pH of about 4.2, a lactic acid bacteria count estimated at 7.6 log<sub>10</sub> cfu/g, and a yeast count estimated at 7.2 log<sub>10</sub> cfu/g [8]. This product

can be considered promising, not just because of the fermentation benefits, but also because of the functional properties reported for the baobab fruit pulp. Fermentation increases the levels of vitamins [9] and antioxidants [10], and the bioavailability of minerals such as iron [11], while baobab fruit pulp brings bioactive compounds such as procyanidins, flavonol glycosides, tiliroside, and ascorbic acid [12–16]. The *Mutchayan* drink can improve the nutritional quality of people's diets. Limited research studies focused on the mechanisms linked to the fermentation process of *Mutchayan*, a principal operation that influences the variability of the fermentation outcomes. Therefore, this study aimed to evaluate, at a laboratory scale, the physico-chemical, microbiological, and antioxidant variations occurring during the fermentation process of *Mutchayan*.

## 2. Materials and Methods

### 2.1. Materials

Red sorghum grains (*Sorghum bicolor* (L.) Moench) purchased at a local market of Abomey-Calavi (Southern Benin, 6°26'51" North and 2°21'31.84" East) and baobab (*Adansonia digitata*) fruit pulp purchased in a local processing unit of baobab fruits, at Bouk-oumbé (Northern Benin, 10°10'36" North and 1°6'22" East), were used for the production of *Mutchayan*.

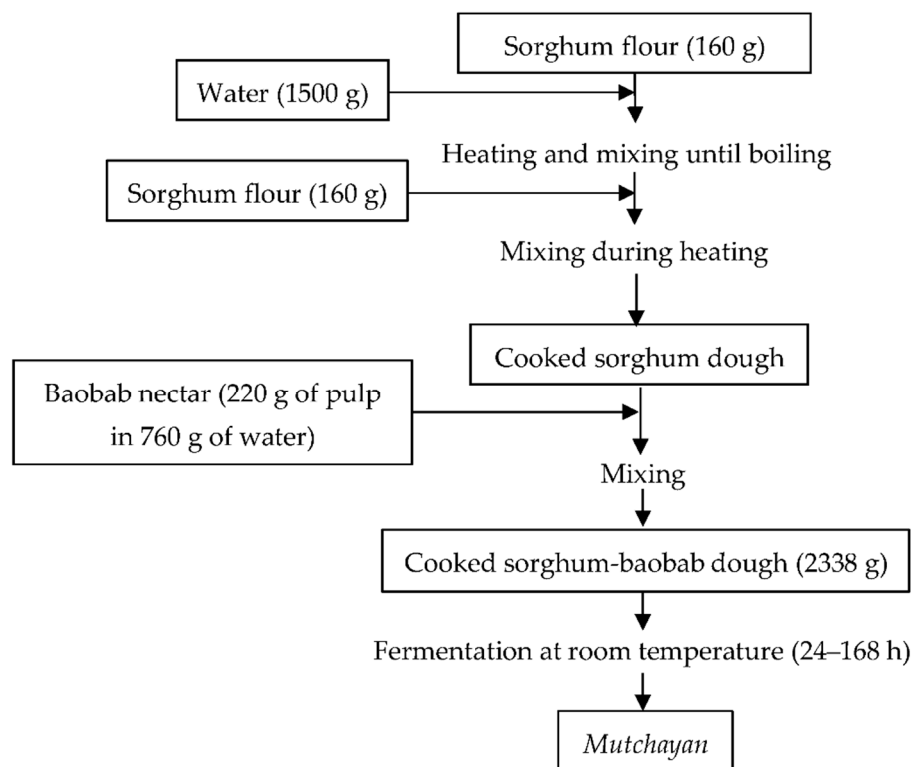
### 2.2. Mutchayan Production and Sampling

*Mutchayan* was produced according to the traditional technology (Figure 1), as described by Chadare et al. [8]. The sorghum grains were sorted and washed with tap water, drained, sundried for two days, and ground into flour. The baobab pulp was sieved using a traditional sieve with a pore size of 0.684 mm; the size was measured through the analysis of images taken by a digital camera (Nikon d90, Nikon CORP., Tokyo, Japan; 4288 × 2848 pixels), using the ImageJ 1.52n software (Wayne Rasband, National Institute of Health, USA). Around 160 g of sorghum grain flour was mixed in 1.5 L of water for making a porridge, which was heated until boiling; then, another 160 g of the flour was added to the boiling porridge and mixed thoroughly to obtain a cooked dough. At the end of the cooking, about 900 mL of diluted baobab fruit pulp (220 g in 760 mL of water) was immediately added to the cooked dough. The mix was cooled, and left to spontaneously ferment at room temperature for 120 h, in a covered plastic bucket; the latter was bought in the local market, cleaned, and rinsed with tap water. During the fermentation process, the temperature was monitored with ibutton devices (temperature data logger; 0–125 °C; MAXIM, Philippines). The production of *Mutchayan* was duplicated, and the sampling was performed in each plastic bucket at 0, 6, 12, 24, 36, 48, 72, 96, and 120 h. The sampling for microbiological analyses was carried out aseptically in a stomacher bag, and the samples ( $n = 18$ ) were analyzed immediately. The samples for physico-chemical analyses ( $n = 18$ ) were collected in small containers, kept at 4 °C, and analyzed within 6–12 h. The parameters assessed were the total viable count (TVC), yeast count (YC), molds count (MC), presumptive lactic acid bacteria count (pLABC), Enterobacteriaceae count (EC), pH, Brix value (BV), total titratable acidity (TTA), dry matter (DM), ascorbic acid content (AAC), and antioxidant capacity (VCEAC). The samples for the antioxidant capacity analysis were stored at −20 °C until the end of the fermentation process, to guarantee the same assessment conditions for all samples.

### 2.3. Physico-Chemical Analyses

The pH of the collected samples was assessed with a pH meter (Cyberscan pH510, EUTECH Instruments, Malaysia), according to ISO 1842:1991 [17], by diluting 10 g of the sample in 20 mL of distilled water. Total titratable acidity (TTA) was assessed according to ISO 750:1998 [18], using the potentiometric method; 10 g of the sample was diluted in distilled water to make 100 mL of suspension which was titrated with 0.1 N sodium hydroxide (NaOH) solution (Carlo Erba reagents, Milan, Italy), using the titrimeter (TITREX 2000, Germany). The total titratable acidity was expressed in grams of lactic acid for 100 g

of the sample, on dry weight basis (g LA/100 g dw). Dry matter content was determined according to AOAC method 2.166 (1980) [19], using an oven (VENTI-Line, VWR, Germany), at 105 °C for 72 h. The Brix value was determined using a digital refractometer 0–54 Bx/1.33–1.42 RI (VWR, Leuven, Belgium), according to ISO 2173:2003 [20].



**Figure 1.** Traditional technology chart of *Mutchayan* production (adapted from Chadare et al. [8]).

## 2.4. Nutritional Analyses

### 2.4.1. Preparation of Extracts

The extraction process, for the ascorbic acid content analysis, was realized according to the method described by Tembo et al. [21], with some modifications. Ten grams (10 g) sample was first diluted in 30 mL of oxalic acid (Merck, Darmstadt, Germany) 2% solution, and the mixture was vortexed for 60 s and centrifuged at 4000 rpm, 20 °C for 10 min. After the centrifuge, the supernatant was taken and filtered using a Whatman paper; the obtained extract was stored at −20 °C until the end of the fermentation follow-up.

The extraction process, for the antioxidant capacity assessment, was performed as follows according to Thaipong et al. [22], with modifications. Two grams (2 g) sample was mixed with 15 mL of 60% methanol solvent in falcon tubes (50 mL). Preliminary works revealed 60% methanol solvent as the adequate mixture (methanol/water) for the maximal extraction of antioxidants from baobab fruit pulp. The mixture was stirred for two hours (2 h) at 37 °C and 200 rpm, using an incubator (GFL 3031, Burgwedel, Germany); then, it was centrifuged at 9000 rpm, 4 °C for 20 min, using the centrifuge MEGA STAR 600R (VWR, Germany).

### 2.4.2. Assessment of Ascorbic Acid Content and Antioxidant Capacity

The ascorbic acid content was determined by the titrimetric method ISO 6557/2:1984 [23], with 2,6-dichlorophenolindophenol (Honeywell, Sneeze, Germany) dyestuff and the ascorbic acid 99–100.5% (VWR chemicals, Leuven, Belgium) as standard. Two (02) milliliters of the extract were titrated with the dyestuff solution, using the titrimeter until a salmon pink coloration persisting for at least 5 s, was obtained. The results were expressed as mg/100 g dw (dry weight) of *Mutchayan*.

The antioxidant capacity was evaluated using a DPPH assay, according to Brand-Williams et al. [24], as described by Thaipong et al. [22]. The stock solution was obtained by mixing 24 mg of DPPH 95% (Alfa Aesar, Kandel, Germany) with 100 mL of methanol 99.9% (HiPerSolv CHROMANORM, VWR chemicals, Leuven, Belgium). The working solution was obtained by mixing 30 mL of the stock solution with 195 mL of methanol to make a DPPH solution of 77.095  $\mu$ M and an absorbance of 1.04, measured at 517 nm, using 6850 UV/Vis spectrophotometer (JENWAY, China). A calibration curve was prepared using the ascorbic acid 99–100.5% standard solutions, at different concentrations (0, 18.46, 92.31, 230.77, 384.62, and 480.77  $\mu$ g/mL); the equation of the calibration curve was  $Y = 0.8147 - 0.0007X$ , with Y the absorbance and X the concentration of ascorbic acid ( $R^2_{adj} = 99.15\%$ ). The results were expressed as mg VCEAC (Vitamin C Equivalent of Antioxidant Capacity) for 100 g of *Mutchayan* (dry weight).

### 2.5. Microbiological Analyses

The general requirements and guidance for microbial examinations [25] were used to prepare the required dilutions and culture media for each sample collected in the two plastic buckets, at the considered time of fermentation. Total flora (TVC) and presumptive lactic acid bacteria (pLAB) were enumerated on Plate Count Agar (PCA) (VWR Chemicals, Leuven, Belgium) and Man Rogosa and Sharpe (MRS) (VWR Chemicals, Leuven, Belgium) media respectively; the Petri dishes were incubated at 30 °C for 72 h according to the standard ISO 4833-1:2013 [26] for total flora (TVC), and according to ISO 15214:1998 [27] for LAB. For yeasts and molds, 100  $\mu$ L of the considered dilution was spread on solidified Sabouraud Dextrose Chloramphenicol Agar medium (VWR Chemicals, Leuven, Belgium), previously poured in the Petri dish, and incubated was performed at 25 °C for 72 to 120 h, according to ISO 21527-2:2008 [28]. Enterobacteriaceae count was assessed by inoculating on Violet Red Bile Glucose (VRBG) medium (VWR Chemicals, Leuven, Belgium) and incubating at 37 °C for 24 h, according to BS ISO 21528-2:2004 [29]. The expression of the results was done according to ISO 7218:2007 [25].

### 2.6. Statistical Analyses

The collected data were analyzed with R 4.0.5, an open-source software program (R Core Team, Vienna, Austria). An analysis of variance was performed to assess the significance of the variability of physico-chemical parameters; the Tukey test was used for the pairwise comparison. Poisson and negative binomial models were used to analyze microbial count data [30,31]; the suitable model considered to analyze each response variable was the one with the lowest Akaike Information Criterion (AIC) value. Then, the Poisson model was used to assess the variability of Enterobacteriaceae count while the negative binomial model was used for Total viable count, yeasts count, and presumptive lactic acid bacteria count. The package “emmeans” was used for pairwise comparisons of count data.

## 3. Results

### 3.1. Physico-Chemical Characteristics of *Mutchayan* during a Spontaneous Fermentation

Table 1 shows that, from 0 h to 72 h, the titratable acidity increased significantly ( $p < 0.05$ ) from  $5.7 \pm 0.1$  to  $6.8 \pm 0.1$  g LA/100 g dw (grams of lactic acid for 100 g of the product (dry weight)), while the pH did not change during the 120 h of fermentation. The dry matter content and the Brix value decreased significantly ( $p < 0.05$ ) between 12 h and 72 h, and between 36 h and 96 h respectively. Indeed, the dry matter decreased from  $18.7 \pm 0.1$  to  $16.7 \pm 0.1$  g/100 g, and the Brix degree decreased from  $6.8 \pm 0.5$  °Bx to  $5.0 \pm 0.0$  °Bx. The ascorbic acid content decreased significantly during the 24 first hours of fermentation from  $91.7 \pm 1.1$  to  $69.8 \pm 4.0$  mg/100 g dw. The antioxidant capacity tended also to decrease to  $657.1 \pm 1.2$  mg VCEAC/100 g (dw) between 0 h and 24 h of fermentation, and tended to increase between 24 h and 120 h, with a final value of  $798.2 \pm 23.2$  mg VCEAC/100 g (dw).

**Table 1.** Physico-chemical characteristics of sorghum flour, baobab pulp, and *Mutchayan* at different times during a spontaneous fermentation.

Products Analyzed	pH	°Brix	TTA (LA g/100 g dw)	DM (g/100 g)	Ascorbic Acid (mg/100 g dw)	Antioxidant Capacity (VCEAC mg/100 g dw)
<i>Mutchayan</i> at 0 h	3.4 ± 0.0 <sup>a</sup>	7.3 ± 0.3 <sup>a</sup>	5.8 ± 0.1 <sup>e,f</sup>	18.7 ± 0.1 <sup>a</sup>	91.7 ± 1.1 <sup>a</sup>	693.5 ± 49.0 <sup>a,b</sup>
<i>Mutchayan</i> at 6 h	3.4 ± 0.0 <sup>a</sup>	7.6 ± 0.1 <sup>a</sup>	5.7 ± 0.1 <sup>f</sup>	18.7 ± 0.3 <sup>a</sup>	84.7 ± 3.8 <sup>a,b</sup>	699.2 ± 51.7 <sup>a,b</sup>
<i>Mutchayan</i> at 12 h	3.3 ± 0.0 <sup>a</sup>	7.5 ± 0.4 <sup>a</sup>	6.0 ± 0.0 <sup>d,e</sup>	18.6 ± 0.2 <sup>a</sup>	79.1 ± 5.0 <sup>a,b</sup>	677.7 ± 0.7 <sup>a,b</sup>
<i>Mutchayan</i> at 24 h	3.3 ± 0.0 <sup>a</sup>	7.4 ± 0.2 <sup>a</sup>	6.1 ± 0.0 <sup>d,e</sup>	18.3 ± 0.0 <sup>b</sup>	69.8 ± 4.0 <sup>b</sup>	657.1 ± 1.2 <sup>b</sup>
<i>Mutchayan</i> at 36 h	3.4 ± 0.0 <sup>a</sup>	6.8 ± 0.5 <sup>a</sup>	6.3 ± 0.0 <sup>c,d</sup>	18.1 ± 0.0 <sup>b</sup>	78.8 ± 8.1 <sup>a,b</sup>	713.8 ± 43.1 <sup>a,b</sup>
<i>Mutchayan</i> at 48 h	3.4 ± 0.0 <sup>a</sup>	6.0 ± 0.4 <sup>a,b</sup>	6.5 ± 0.1 <sup>a,b,c</sup>	17.5 ± 0.0 <sup>c</sup>	80.8 ± 1.1 <sup>a,b</sup>	802.9 ± 48.9 <sup>a</sup>
<i>Mutchayan</i> at 72 h	3.3 ± 0.0 <sup>a</sup>	6.2 ± 0.9 <sup>a,b</sup>	6.8 ± 0.1 <sup>a</sup>	16.9 ± 0.0 <sup>d</sup>	76.6 ± 0.9 <sup>a,b</sup>	766.1 ± 0.0 <sup>a,b</sup>
<i>Mutchayan</i> at 96 h	3.3 ± 0.0 <sup>a</sup>	5.0 ± 0.0 <sup>b</sup>	6.6 ± 0.1 <sup>a,b</sup>	16.9 ± 0.1 <sup>d</sup>	79.3 ± 0.0 <sup>a,b</sup>	751.5 ± 17.9 <sup>a,b</sup>
<i>Mutchayan</i> at 120 h	3.4 ± 0.1 <sup>a</sup>	5.1 ± 0.4 <sup>b</sup>	6.3 ± 0.1 <sup>b,c,d</sup>	16.7 ± 0.1 <sup>d</sup>	82.6 ± 3.9 <sup>a,b</sup>	798.2 ± 23.2 <sup>a</sup>
Sorghum flour	6.1 ± 0.0	8.2 ± 0.3	0.6 ± 0.0	91.3 ± 0.1	0.0 ± 0.0	NA
Baobab pulp	3.1 ± 0.0	73.4 ± 1.7	14.4 ± 0.1	87.3 ± 0.5	299.3 ± 4.4	3004.6 ± 1.6

The mean values ( $n = 2$ ) with different letters in a column are significantly different at the 5% threshold. TTA: Total titratable acidity; LA: Lactic acid; DM: Dry matter; NA: Not available.

### 3.2. Microbial Changes during *Mutchayan* Fermentation

Different morphotypes of yeast were observed during the fermentation: large yeast colonies (LY), and small yeast colonies (SY) (Figure 2). The considered small yeast colonies were circular and whitish while the considered large yeast colonies were circular and greyish. The molds observed were whitish filamentous fungi.



Legend: SY: Small yeast; LY: Large yeast; M: Molds.

**Figure 2.** Yeast and mold morphotypes in *Mutchayan*.

At the beginning of the fermentation (0 h), while the total viable count was estimated at  $3.5 \times 10^3 \pm 2.3 \times 10^3$  cfu/g (Table 2), presumptive lactic acid bacteria, yeasts, and Enterobacteriaceae were not counted (Table 3); they were observed after 12 h of fermentation. Between 0 h and 6 h, the dough temperature was decreasing from  $57.8 \pm 3.9$  °C to  $30.5 \pm 0.0$  °C. Molds were observed at 0 h, with a population estimated at 500 cfu/g; after an increase between 24 and 36 h, where their load reached a maximum estimated value of  $8 \times 10^6 \pm 0.0$  cfu/g (Table 2), molds growth tended to be inhibited and the load decreased below  $4 \times 10^6$  cfu/g at the end of the fermentation. The total viable count increased significantly ( $p < 0.05$ ) from 12 to 36 h, reaching  $6.9 \times 10^7 \pm 5.6 \times 10^7$  cfu/g and remained constant until 120 h, with a dough temperature ranging from 28.0 to 32.3 °C (Table 2).



**Table 2.** Enterobacteriaceae, molds, and total viable counts (in cfu/g of *Mutchayan* sample) during *Mutchayan* fermentation.

<i>Mutchayan</i> at	TVC	MC	EC
0 h	$3.5 \times 10^3 \pm 2.3 \times 10^3$ c	<b>500 ± 0</b>	<10
6 h	$4.2 \times 10^3 \pm 2.5 \times 10^3$ c	1000 ± 0	<10
12 h	$6.6 \times 10^3 \pm 0.8 \times 10^3$ c	<b>500 ± 0</b>	<b>50 ± 14</b> b
24 h	$8.4 \times 10^6 \pm 9.9 \times 10^6$ b	<4 × 10 <sup>6</sup>	<10
36 h	$6.9 \times 10^7 \pm 5.6 \times 10^7$ a	<b>8 × 10<sup>6</sup> ± 0.0</b>	115 ± 7 a
48 h	$4.7 \times 10^7 \pm 0.6 \times 10^7$ a	<4 × 10 <sup>5</sup>	<10
72 h	$10.5 \times 10^7 \pm 3.7 \times 10^7$ a	<4 × 10 <sup>6</sup>	NA
96 h	$14.6 \times 10^7 \pm 5.8 \times 10^7$ a	<b>4 × 10<sup>6</sup> ± 0.0</b>	NA
120 h	$14.2 \times 10^7 \pm 2.3 \times 10^7$ a	<4 × 10 <sup>6</sup>	NA

The mean values ( $n = 2$ ) with different letters in a column are significantly different at the 5% threshold. The values in bold represented the estimated count of microorganisms (number of observed colonies ranged between 4 and 10); TVC: Total viable count; MC: Molds count; EC: Enterobacteriaceae count; NA: Not available.

**Table 3.** Yeasts and presumptive lactic acid bacteria counts (in cfu/g of *Mutchayan* sample) during *Mutchayan* fermentation.

<i>Mutchayan</i> at	YC	SYC	LYC	pLABC
<i>Mutchayan</i> at 0 h	<100	<100	<100	<10
<i>Mutchayan</i> at 6 h	<100	<100	<100	<10
<i>Mutchayan</i> at 12 h	$6.5 \times 10^2 \pm 7.8 \times 10^2$ d	$6.5 \times 10^2 \pm 7.8 \times 10^2$ d	<100	$2.4 \times 10^2 \pm 3.0 \times 10^2$ d
<i>Mutchayan</i> at 24 h	$10.2 \times 10^6 \pm 7.7 \times 10^5$ c	$9.6 \times 10^6 \pm 6.4 \times 10^3$ c	<b><math>1.2 \times 10^6 \pm 0.0</math> c</b>	$4.6 \times 10^6 \pm 5.3 \times 10^6$ c
<i>Mutchayan</i> at 36 h	$2.3 \times 10^7 \pm 0.7 \times 10^7$ b	$2.1 \times 10^7 \pm 0.5 \times 10^7$ b,c	$2.5 \times 10^6 \pm 0.0$ b,c	$2.6 \times 10^7 \pm 1.2 \times 10^7$ b
<i>Mutchayan</i> at 48 h	$7.9 \times 10^7 \pm 2.2 \times 10^7$ a	$7.0 \times 10^7 \pm 0.9 \times 10^7$ a,b	$1.9 \times 10^7 \pm 0.0$ a	$5.7 \times 10^7 \pm 0.3 \times 10^7$ a,b
<i>Mutchayan</i> at 72 h	$8.9 \times 10^7 \pm 5.1 \times 10^7$ a	$8.6 \times 10^7 \pm 5.5 \times 10^7$ a	<b><math>3.7 \times 10^6 \pm 3.8 \times 10^6</math> b,c</b>	$6.4 \times 10^7 \pm 4.1 \times 10^7$ a,b
<i>Mutchayan</i> at 96 h	$8.1 \times 10^7 \pm 0.8 \times 10^7$ a	$7.5 \times 10^7 \pm 0.1 \times 10^7$ a,b	$1.4 \times 10^7 \pm 0.0$ a,b	$11.2 \times 10^7 \pm 0.8 \times 10^7$ a
<i>Mutchayan</i> at 120 h	$10.1 \times 10^7 \pm 1.3 \times 10^7$ a	$9.2 \times 10^7 \pm 2.5 \times 10^7$ a	$4.1 \times 10^7 \pm 0.0$ a	$13.0 \times 10^7 \pm 0.8 \times 10^7$ a

The mean values ( $n = 2$ ) with different letters in a column are significantly different at the 5% threshold. The values in bold represented the estimated count of microorganisms (number of observed colonies ranged between 4 and 10); YC: Yeast Count; SYC: Small Yeast count; LYC: Large Yeast Count; pLABC: presumptive Lactic Acid Bacteria Count.

Yeast count increased significantly ( $p < 0.05$ ) (Table 3) during the fermentation process, from  $6.5 \times 10^2 \pm 7.8 \times 10^2$  cfu/g, at 12 h, to  $7.9 \times 10^7 \pm 2.2 \times 10^7$  cfu/g at 48 h of fermentation; only small yeasts colonies were detected at 12 h of fermentation. A significant increase in yeast population was observed from 12 to 24 h, for small yeast colonies, and large yeast colonies as well. The small yeast count (SYC) stayed the same after 48 h of fermentation, while the large yeasts count remained the same after 96 h of fermentation (Table 3). Similarly to yeasts, presumptive lactic acid bacteria count (pLABC) increased between 12 and 36 h from  $2.4 \times 10^2 \pm 3.0 \times 10^2$  to  $2.6 \times 10^7 \pm 1.2 \times 10^7$  cfu/g. After 48 h, the pLABC did not change significantly (Table 3).

The Enterobacteriaceae count increased from <10 to  $50 \pm 14$  cfu/g between 6 and 12 h, and decreased from  $50 \pm 14$  to <10 cfu/g between 12 and 24 h (Table 2). After 36 h of fermentation, similar observations were made; the Enterobacteriaceae load increased from <10 to  $115 \pm 7$  cfu/g, and later decreased to <10 cfu/g. Enterobacteriaceae growth is inhibited during the fermentation of *Mutchayan*.

#### 4. Discussion

Yeasts and presumptive lactic acid bacteria are the main microorganisms counted during the fermentation of *Mutchayan*. During the first 12 h of the fermentation, the microbial activity was limited and no significant change was observed in physico-chemical parameters until 24 h. After 24 h of fermentation, the total microbial flora and the presumptive lactic acid bacteria load increased (about  $10^6$  cfu/g). That increase might contribute to the production of several organic acids like propionic, formic, lactic, and acetic acids [32], increasing the titratable acidity from 36 to 120 h. Despite the increase of the titratable acidity, the amount of acid produced was not sufficient to decrease the already acidic pH of

*Mutchayan* (pH = 3.4 at 0 h of fermentation) as observed for the fermentation of cereal-based products as *Mawè* [33] and *Gowé* [7], where the pH decreased from 6.1–6.2 to 3.5–3.6 [33], almost the pH of *Mutchayan* at 0 h of fermentation.

The non-detection of LAB and yeasts at the beginning of *Mutchayan* fermentation could be linked to the cooking process applied and the relatively high temperature of the dough during the first 4 h (58 to 41 °C). However, the presence of the molds at the beginning of the fermentation process is probably related to the molds' ability to survive in critical conditions. Some mold species can grow at low pH values (pH = 3.2) and low water activity [34]; other species such as *Neosartorya fumigata*, *Neosartorya fischeri*, and *Byssoschlamys nivea*, can survive at pasteurization temperatures above 60 °C and produce spores [35]. The inhibition of mold growth along the fermentation process could be linked to the LAB and yeast activities. It has been proven that some strains of *Lactobacillus* sp. have a strong inhibitory capacity on fungal growth; that is the case of *Lactobacillus plantarum* strain ITEM 17215, which inhibited the growth of mycotoxigenic fungi as *Aspergillus flavus* [36,37]. Some yeast strains were also effective against green molds; this is the case with *Pichia kudriavzevii*, *Kluyveromyces marxianus*, and *Issatchenkia orientali* [38].

As the fermentation duration increased, yeasts and presumptive lactic acid bacteria counts became higher (6–8 log<sub>10</sub> cfu/g) than at the beginning, inducing a significant increase in antioxidant capacity; further studies are required to establish the relationship of these microorganisms with the increasing of antioxidant capacity. The association of LAB and yeasts were found to be responsible for *Mutchayan* fermentation, which agrees with the results of Chadare et al. [8] and other studies of indigenous cereal-based fermented foods, such as Kenyan *Busaa*, *Kaffir* beer, Nigerian *Ogi*, *Pito*, and *Sekete* [39].

The occurrence of Enterobacteriaceae in *Mutchayan* could be linked to unmastered surrounding conditions (the room fermentation potentially contaminated by people), and the fermenter (plastic bucket). The absence of an air-locker system on the fermenter (traditional method) could favor the airflow and migration of very small particles and microorganisms from outside to inside the fermenter. Similar trends were reported in the processing of other products like *Gowé* [40], and *Calugi*, [41]. During *Mutchayan* fermentation, the decrease in Enterobacteriaceae load could be due to the low pH values and the increase of the presumptive lactic acid bacteria activity, since it was reported that LAB produces bacteriocins, controlling the pathogenic microorganisms' growth [42,43].

The outcomes of a fermentation process are affected by many factors and conditions such as the nutrients content, the concentration in oxygen, the temperature, the duration, the room temperature, or the house microbiota [11,44]. The modification of the processing conditions described in this manuscript can affect the dynamic of yeasts, lactic acid bacteria and molds during the fermentation process of *Mutchayan*.

## 5. Conclusions

The fermentation of *Mutchayan* can be characterized by a domination of molds during the first 12 h. Their growth was inhibited by the development of yeast and presumptive lactic acid bacteria, which dominated the fermentation. Along these microbial changes, a decrease in Brix value, dry matter, and an increase in titratable acidity and antioxidant capacity, were observed during fermentation. Further investigations are needed for assessing the effects of different fermentation conditions on the microbial dynamic, and identifying the different microbial species and their specific role in the fermentation of *Mutchayan*.

**Author Contributions:** Conceptualization, A.M.G. and F.J.C.; methodology, A.M.G., F.J.C., S.W.P.; C.O.A.; software, A.M.G.; validation, F.J.C. and D.J.H., formal analysis, C.O.A. and A.M.G.; data curation, A.M.G.; writing—original draft preparation, A.M.G.; writing—review and editing, A.M.G., F.J.C., S.W.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by MasterCard Foundation, grant number RU/2018/CARP+/01.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** This work was supported by the MasterCard Foundation via the RUFORUM “Community Action Research Program Plus” CARP + grant (RU/2018/CARP+/01).

**Conflicts of Interest:** The authors declare there is no conflicts of interest.

## References

- Ross, R.P.; Morgan, S.; Hill, C. Preservation and fermentation: Past, present and future. *Int. J. Food Microbiol.* **2002**, *79*, 3–16. [[CrossRef](#)] [[PubMed](#)]
- Liu, X.; Narbad, A. Lactic Acid Bacteria-Based Foods Fermentations. In *Lactic Acid Bacteria in Foodborne Hazards Reduction*; Springer, Ed.; Springer: Singapore, 2018; pp. 141–181.
- Katina, K.; Arendt, E.; Liukkonen, K.-H.; Autio, K.; Flander, L.; Poutanen, K. Potential of sourdough for healthier cereal products. *Trends Food Sci. Technol.* **2005**, *16*, 104–112. [[CrossRef](#)]
- Wacoo, A.P.; Mukisa, I.M.; Meeme, R.; Byakika, S.; Wendi, D.; Sybesma, W.; Kort, R. Probiotic Enrichment and Reduction of Aflatoxins in a Traditional African Maize-Based Fermented Food. *Nutrients* **2019**, *11*, 265. [[CrossRef](#)] [[PubMed](#)]
- Hounhouigan, D.J.; Nout, M.J.R.; Nago, C.M.; Houben, J.H. Composition and microbiological and physical attributes of mawè, a fermented maize dough from Benin. *Int. J. Food Sci. Technol.* **1993**, *28*, 513–517. [[CrossRef](#)]
- Nago, M.C.; Hounhouigan, J.D.; Zanou, E.; Akissoe, N.; Mestres, C. Characterization of the Beninese traditional ogi, a fermented maize slurry: Physicochemical and microbiological aspects. *Int. J. Food Sci. Technol.* **1998**, *33*, 307–315. [[CrossRef](#)]
- Michodjehoun-Mestres, L.; Hounhouigan, D.J.; Dossou, J.; Mestres, C. Physical, chemical and microbiological changes during natural fermentation of “gowé”, a sprouted or non sprouted sorghum beverage from West-Africa. *Afr. J. Biotechnol.* **2005**, *4*, 487–496.
- Chadare, F.J.; Gayet, D.P.; Azokpota, P.; Nout, M.J.R.; Linnemann, A.R.; Hounhouigan, J.D.; Van Boekel, M.A.J.S. Three Traditional Fermented Baobab Foods from Benin, Mutchayan, Dikouanyouri, and Tayohounta: Preparation, Properties, and Consumption. *Ecol. Food Nutr.* **2010**, *49*, 279–297. [[CrossRef](#)]
- El Sheikh, A.F.; Montet, D. African Fermented Foods: Historical Roots and Real Benefits. In *Microorganisms and Fermentation of Traditional Foods*; Ray, R.C., Montet, D., Eds.; CRC Press: Boca Raton, FL, USA, 2014; pp. 188–222.
- Adebo, O.A.; Njobeh, P.B.; Kayitesi, E. Fermentation by *Lactobacillus fermentum* strains (singly and in combination) enhances the properties of ting from two whole grain sorghum types. *J. Cereal Sci.* **2018**, *82*, 49–56. [[CrossRef](#)]
- Montet, D.; Ray, R.C.; Zakhia-Rozis, N. Lactic Acid Fermentation of Vegetables and Fruits. In *Microorganisms and Fermentation of Traditional Foods*; Ray, R.C., Montet, D., Eds.; CRC Press: Boca Raton, FL, USA, 2014; p. 392.
- Chadare, F.J.; Linnemann, A.R.; Hounhouigan, J.D.; Nout, M.J.R.; van Boekel, M.A.J.S. Baobab Food Products: A review on their Composition and Nutritional Value. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 254–274. [[CrossRef](#)]
- Braca, A.; Sinisgalli, C.; De Leo, M.; Muscatello, B.; Cioni, P.L.; Milella, L.; Ostuni, A.; Giani, S.; Sanogo, R. Phytochemical Profile, Antioxidant and Antidiabetic Activities of *Adansonia digitata* L. (Baobab) from Mali, as a Source of Health-Promoting Compounds. *Molecules* **2018**, *23*, 3104. [[CrossRef](#)]
- RRusso, M.; Ronci, M.B.; Vilmercati, A.; Gionfriddo, M.; Fanali, C.; Dugo, L.; Locato, V.; Mondello, L.; De Gara, L. African baobab (*Adansonia digitata*) fruit as promising source of procyanidins. *Eur. Food Res. Technol.* **2019**, *246*, 297–306. [[CrossRef](#)]
- Ismail, B.B.; Pu, Y.; Guo, M.; Ma, X.; Liu, D. LC-MS/QTOF identification of phytochemicals and the effects of solvents on phenolic constituents and antioxidant activity of baobab (*Adansonia digitata*) fruit pulp. *Food Chem.* **2019**, *277*, 279–288. [[CrossRef](#)] [[PubMed](#)]
- Sokeng, A.J.T.; Sobolev, A.P.; Di Lorenzo, A.; Xiao, J.; Mannina, L.; Capitani, D.; Daglia, M. Metabolite characterization of powdered fruits and leaves from *Adansonia digitata* L. (baobab): A multi-methodological approach. *Food Chem.* **2018**, *272*, 93–108. [[CrossRef](#)] [[PubMed](#)]
- ISO. *Fruits and Vegetables Products—Determination of Ph*; ISO: Geneva, Switzerland, 1991.
- ISO. *Fruits and Vegetables Products—Determination of Titratable Acidity*; ISO: Geneva, Switzerland, 1998.
- AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed.; Association of Official Analytical Chemists: Washington DC, USA, 1980; p. 1040.
- ISO. *Fruit and Vegetable Products—Determination of Soluble Solids—Refractometric Method*; ISO: Geneva, Switzerland, 2003.
- Tembo, D.T.; Holmes, M.J.; Marshall, L.J. Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (*Adansonia digitata*) pulp from Malawi. *J. Food Compos. Anal.* **2017**, *58*, 40–51. [[CrossRef](#)]
- Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Hawkins Byrne, D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* **2006**, *19*, 669–675. [[CrossRef](#)]
- ISO. *Fruits, Vegetables and Derived Products—Determination of Acid Ascorbic Content—Part 2: Routine Methods*; ISO: Geneva, Switzerland, 1984.
- Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30. [[CrossRef](#)]



25. ISO. *Microbiology of Food and Animal Feeding of Stuffs—General Requirements and Guidance for Microbiological Examinations*; ISO: Geneva, Switzerland, 2007.
26. ISO. *Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms, in Part 1: Colony Count at 30°C by the Pour Plate Technique*; ISO: Geneva, Switzerland, 2013.
27. ISO. *Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Mesophilic Lactic Acid Bacteria—Colony Count Technique at 30 °C*; ISO: Geneva, Switzerland, 1998.
28. ISO. *Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—, in Part 2: Colony Count Technique in Products with Water Activity Less than or Equal to 0,95*; ISO: Geneva, Switzerland, 2008.
29. ISO. *Microbiology of Food and Animal Feeding Stuffs—Horizontal Methods for the Detection and Enumeration of Enterobacteriaceae in Part 2: Colony Count Method*; ISO: Geneva, Switzerland, 2004.
30. Zeileis, A.; Kleiber, C.; Jackman, S. Regression Models for Count Data in R. *J. Stat. Softw.* **2008**, *27*, 25. [\[CrossRef\]](#)
31. Muoka, A.K.; Ngesa, O.O.; Waititu, A.G. Statistical Models for Count Data. *Sci. J. Appl. Math. Stat.* **2016**, *4*, 256. [\[CrossRef\]](#)
32. Bangar, S.P.; Suri, S.; Trif, M.; Ozogul, F. Organic acids production from lactic acid bacteria: A preservation approach. *Food Biosci.* **2022**, *46*, 101615. [\[CrossRef\]](#)
33. Hounhouigan, D.; Nout, M.; Nago, C.; Houben, J.; Rombouts, F. Changes in the Physico-Chemical Properties of Maize During Natural Fermentation of Mawè. *J. Cereal Sci.* **1993**, *17*, 291–300. [\[CrossRef\]](#)
34. Racchi, I.; Scaramuzza, N.; Hidalgo, A.; Berni, E. Combined effect of water activity and pH on the growth of food-related ascospore-forming molds. *Ann. Microbiol.* **2020**, *70*, 69. [\[CrossRef\]](#)
35. Santos, J.L.P.D.; Samapundo, S.; Biyikli, A.; van Impe, J.; Akkermans, S.; Höfte, M.; Abatih, E.N.; Sant'Ana, A.S.; Devlieghere, F. Occurrence, distribution and contamination levels of heat-resistant moulds throughout the processing of pasteurized high-acid fruit products. *Int. J. Food Microbiol.* **2018**, *281*, 72–81. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Quattrini, M.; Bernardi, C.; Stuknytė, M.; Masotti, F.; Passera, A.; Ricci, G.; Vallone, L.; De Noni, I.; Brasca, M.; Fortina, M.G. Functional characterization of *Lactobacillus plantarum* ITEM 17215: A potential biocontrol agent of fungi with plant growth promoting traits, able to enhance the nutritional value of cereal products. *Food Res. Int.* **2018**, *106*, 936–944. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Rizzello, C.G.; Lorusso, A.; Montemurro, M.; Gobbetti, M. Use of sourdough made with quinoa (*Chenopodium quinoa*) flour and autochthonous selected lactic acid bacteria for enhancing the nutritional, textural and sensory features of white bread. *Food Microbiol.* **2016**, *56*, 1–13. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Delali, K.I.; Chen, O.; Wang, W.; Yi, L.; Deng, L.; Zeng, K. Evaluation of yeast isolates from kimchi with antagonistic activity against green mold in citrus and elucidating the action mechanisms of three yeast: *P. kudriavzevii*, *K. marxianus*, and *Y. lipolytica*. *Postharvest Biol. Technol.* **2021**, *176*, 111495. [\[CrossRef\]](#)
39. Gotcheva, V.; Pandiella, S.S.; Angelov, A.; Roshkova, Z.; Webb, C. MMonitoring the fermentation of the traditional Bulgarian beverage boza. *Int. J. Food Sci. Technol.* **2001**, *36*, 129–134. [\[CrossRef\]](#)
40. Adinsi, L.; Mestres, C.; Akissoé, N.; Vieira-Dalodé, G.; Anihouvi, V.; Durand, N.; Hounhouigan, D.J. Comprehensive quality and potential hazards of gowe, a malted and fermented cereal beverage from West Africa. A diagnostic for a future re-engineering. *Food Control* **2017**, *82*, 18–25. [\[CrossRef\]](#)
41. Miguel, M.G.d.P.; Santos, M.R.R.M.; Duarte, W.F.; de Almeida, E.G.; Schwan, R.F. Physico-chemical and microbiological characterization of corn and rice ‘calugi’ produced by Brazilian Amerindian people. *Food Res. Int.* **2012**, *49*, 524–532. [\[CrossRef\]](#)
42. Assouhoun-Djeni, N.; Messaoudi, S.; Lhomme, E.; Koussemon-Camara, M.; Ouassa, T.; Chobert, J.-M.; Onno, B.; Dousset, X. Biodiversity, dynamics and antimicrobial activity of lactic acid bacteria involved in the fermentation of maize flour for doklu production in Côte d'Ivoire. *Food Control* **2016**, *62*, 397–404. [\[CrossRef\]](#)
43. Onda, T.; Yanagida, F.; Tsuji, M.; Shinohara, T.; Yokotsuka, K. Time series analysis of aerobic bacterial flora during Miso fermentation. *Lett. Appl. Microbiol.* **2003**, *37*, 162–168. [\[CrossRef\]](#)
44. Catzeddu, P. Chapter 14—Sourdough Breads. In *Flour and Breads and Their Fortification in Health and Disease Prevention (Second Edition)*; Preedy, V.R., Watson, R.R., Eds.; Academic Press: Cambridge, MA, USA, 2019; pp. 177–188.

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.