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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 †

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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

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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 *Mawè* [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 $\log_{10}$ cfu/g, and a yeast count estimated at 7.2 $\log_{10}$ 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 *Boukoumbé* (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].

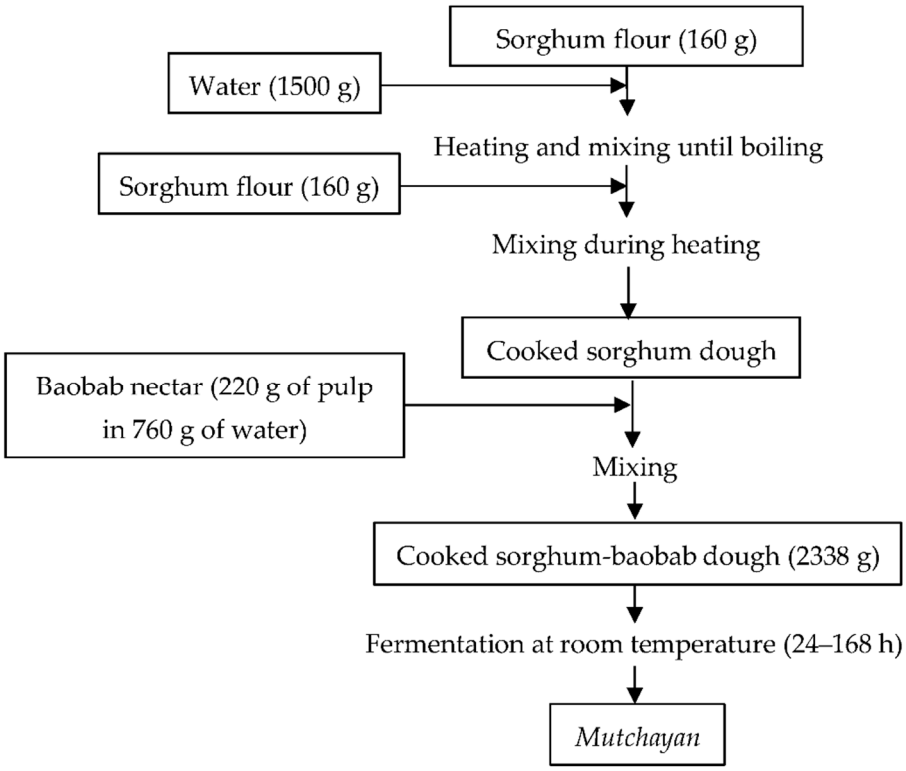


Figure 1. Traditional technology chart of *Mutchayan* production (adapted from Chadare et al. [8]).

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, Sleeze, 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 µM 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 µg/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^2adj = 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 µL 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 ± 0.1 to 6.8 ± 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 ± 0.1 to 16.7 ± 0.1 g/100 g, and the Brix degree decreased from 6.8 ± 0.5 °Bx to 5.0 ± 0.0 °Bx. The ascorbic acid content decreased significantly during the 24 first hours of fermentation from 91.7 ± 1.1 to 69.8 ± 4.0 mg/100 g dw. The antioxidant capacity tended also to decrease to 657.1 ± 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 ± 23.2 mg VCEAC/100 g (dw).

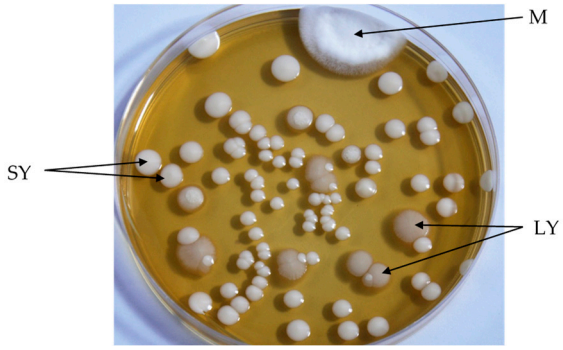
Table 1. Physico-chemical characteristics of sorghum flour, baobab pulp, and *Mutchayan* at different times during a spontaneous fermentation.

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)
Mutchayan at 0 h	3.4 ± 0.0 ^{a}	7.3 ± 0.3 ^{a}	5.8 ± 0.1 $^{e.f}$	18.7 ± 0.1 ^{a}	91.7 ± 1.1 ^{a}	693.5 ± 49.0 $^{a.b}$
Mutchayan at 6 h	3.4 ± 0.0 ^{a}	7.6 ± 0.1 ^{a}	5.7 ± 0.1 ^{f}	18.7 ± 0.3 ^{a}	84.7 ± 3.8 $^{a.b}$	699.2 ± 51.7 $^{a.b}$
Mutchayan at 12 h	3.3 ± 0.0 ^{a}	7.5 ± 0.4 ^{a}	6.0 ± 0.0 $^{d.e}$	18.6 ± 0.2 ^{a}	79.1 ± 5.0 $^{a.b}$	677.7 ± 0.7 $^{a.b}$
Mutchayan at 24 h	3.3 ± 0.0 ^{a}	7.4 ± 0.2 ^{a}	6.1 ± 0.0 $^{d.e}$	18.3 ± 0.0 ^{b}	69.8 ± 4.0 ^{b}	657.1 ± 1.2 ^{b}
Mutchayan at 36 h	3.4 ± 0.0 ^{a}	6.8 ± 0.5 ^{a}	6.3 ± 0.0 $^{c.d}$	18.1 ± 0.0 ^{b}	78.8 ± 8.1 $^{a.b}$	713.8 ± 43.1 $^{a.b}$
Mutchayan at 48 h	3.4 ± 0.0 ^{a}	6.0 ± 0.4 $^{a.b}$	6.5 ± 0.1 $^{a.b.c}$	17.5 ± 0.0 ^{c}	80.8 ± 1.1 $^{a.b}$	802.9 ± 48.9 ^{a}
Mutchayan at 72 h	3.3 ± 0.0 ^{a}	6.2 ± 0.9 $^{a.b}$	6.8 ± 0.1 ^{a}	16.9 ± 0.0 ^{d}	76.6 ± 0.9 $^{a.b}$	766.1 ± 0.0 $^{a.b}$
Mutchayan at 96 h	3.3 ± 0.0 ^{a}	5.0 ± 0.0 ^{b}	6.6 ± 0.1 $^{a.b}$	16.9 ± 0.1 ^{d}	79.3 ± 0.0 $^{a.b}$	751.5 ± 17.9 $^{a.b}$
Mutchayan at 120 h	3.4 ± 0.1 ^{a}	5.1 ± 0.4 ^{b}	6.3 ± 0.1 $^{b.c.d}$	16.7 ± 0.1 ^{d}	82.6 ± 3.9 $^{a.b}$	798.2 ± 23.2 ^{a}
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 ± 3.9 °C to 30.5 ± 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 4×10^6 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.

***Mutchayan* at**	**TVC**	**MC**	**EC**
0 h	$3.5 \times 10^3 \pm 2.3 \times 10^3$ c	**500 ± 0**	<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	**500 ± 0**	**50 ± 14** b
24 h	$8.4 \times 10^6 \pm 9.9 \times 10^6$ b	$<4 \times 10^6$	<10
36 h	$6.9 \times 10^7 \pm 5.6 \times 10^7$ a	$\mathbf{8 \times 10^6 \pm 0.0}$	115 ± 7 a
48 h	$4.7 \times 10^7 \pm 0.6 \times 10^7$ a	$<4 \times 10^5$	<10
72 h	$10.5 \times 10^7 \pm 3.7 \times 10^7$ a	$<4 \times 10^6$	NA
96 h	$14.6 \times 10^7 \pm 5.8 \times 10^7$ a	$\mathbf{4 \times 10^6 \pm 0.0}$	NA
120 h	$14.2 \times 10^7 \pm 2.3 \times 10^7$ a	$<4 \times 10^6$	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.

***Mutchayan* at**	**YC**	**SYC**	**LYC**	**pLABC**
Mutchayan at 0 h	<100	<100	<100	<10
Mutchayan at 6 h	<100	<100	<100	<10
Mutchayan 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
Mutchayan 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	$\mathbf{1.2 \times 10^6 \pm 0.0}$ c	$4.6 \times 10^6 \pm 5.3 \times 10^6$ c
Mutchayan 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
Mutchayan 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
Mutchayan 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	$\mathbf{3.7 \times 10^6 \pm 3.8 \times 10^6}$ b,c	$6.4 \times 10^7 \pm 4.1 \times 10^7$ a,b
Mutchayan 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
Mutchayan 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 ± 14 cfu/g between 6 and 12 h, and decreased from 50 ± 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 ± 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 *Byssochlamys 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 $\log_{10}$ 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*.

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