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Microbial Succession and Variation of Physico-Chemical Characteristics and Antioxidant Capacity during Spontaneous Fermentation of *Mutchayan*, A Traditional Fermented Baobab Derived Food †

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Abstract: *Mutchayan* is a traditionally fermented cooked cereal dough mixed to baobab pulp, consumed in Benin. The present study evaluated the physico-chemical and microbiological changes of this product during 0 to 120 h of spontaneous fermentation. Analysis of the fermentation process revealed on one hand an increase of lactic acid and antioxidant capacity and a decrease of ascorbic acid content. Dry matter content and Brix value decreased while pH did not significant change. Microbiological analysis revealed the presence of molds at the beginning of the fermentation, which growth was inhibited after 36 h, while the LAB and yeasts dominated the process.

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

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1. Introduction

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

Several products are derived from cereals acid fermentation: *Mawè*, [5], *Ogi* [6], derived from maize, *Gowé* [7] derived from sorghum, *Mutchayan* [8] derived from cereals (maize or sorghum or millet). *Mutchayan*, a traditional food of "Otamari" socio-cultural group of northern Benin, derives from the fermentation in a jar of cooked cereal dough, mixed with diluted baobab (*Adansonia digitata*) fruit pulp, from 24 to 168 h. It is characterized by a pH of about 4.2, lactic acid bacteria and yeast counts estimated at 7.6 $\log_{10}$ cfu/g and 7.2 $\log_{10}$ cfu/g respectively [8]. *Mutchayan* can be considered as promising, not only because of the fermentation benefits, but also the functional properties reported for the baobab fruit pulp. Therefore, the present study aimed to evaluate the physico-chemical, microbiological and nutritional changes occurring during the fermentation process.

2. Materials and Methods

2.1. Materials

Red sorghum grains (*Sorghum bicolor* (L.) Moench) purchased on a local market of *Abomey-Calavi* (southern Benin), and baobab (*Adansonia digitata*) fruit pulp purchased in a local processing unit of baobab fruits, at *Boukoumbé* (northern Benin), were used for the production of *Mutchayan*.

2.2. Mutchayan Production and Sampling

Mutchayan was produced based on the traditional technology [8]. Around of 160 g of sorghum grains flour was mixed in 1.5 L of water for making a porridge which was heated till boiling; then, 160 g of the flour was added to the porridge and mixed thoroughly. 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 let to spontaneously ferment at room temperature during 120 h, in a covered plastic bucket. Along this process, the temperature was monitored with ibutton devices (temperature data logger), and samples were collected at 0, 6, 12, 24, 36, 48, 72, 96 and 120 h to assess the total viable count (TVC), yeast count (YC), molds count (MC), lactic acid bacteria count (LABC), Enterobacteriaceae count (EC), pH, Brix value (BV), total titratable acidity (TTA), dry matter (DM), ascorbic acid content (AAC) and antioxidant capacity (VCEAC).

2.3. Nutritional and Physico-Chemical Analyses

The extraction process for the antioxidant capacity was performed as follows according to Thaiponget al. [9], with modifications. Two grams (02 g) sample was mixed to 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 antioxidant capacity was evaluated using DPPH assay, according to Brand-Williams et al. [10], as described by Thaipong et al. [9], with some modifications. The results were expressed as mg VCEAC (Vitamin C Equivalent of Antioxidant Capacity) for 100 g of the product (dry weight).

The ascorbic acid content was determined by titrimetric method ISO 6557/2:1984; the results were expressed as mg/100 mg dw (dry weight) of *Mutchayan* samples.

The pH of the collected samples was assessed with a pH meter, according to ISO 1842:1991. Total titratable acidity (TTA) was assessed according to ISO 750:1998, 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 hydroxide sodium (NaOH) solution. Dry matter content was determined according to AOAC method 2.166 (1980). The Brix value was determined using a digital refractometer, according to ISO 2173:2003.

2.4. Microbiological Analyses

The general requirements and guidance for microbial examinations (ISO 7218:2007) were used to prepare required dilutions and culture media. Total flora and lactic acid bacteria (LAB) were enumerated on Plate Count Agar (PCA) and Man Rogosa and Sharpe (MRS) media respectively; the Petri dishes were incubated at 30 °C for 72 h according to the standard ISO 4833-1:2013 for total flora (TVC), and according to ISO 15214:1998 for LAB. For yeasts and molds, 100 µL of the considered dilution was surface inoculated on solidified Sabouraud Dextrose Chloramphenicol Agar medium; incubation was performed at 25 °C for 72 to 120 h, according to ISO 21527-2:2008. Enterobacteriaceae count was assessed by inoculating on Violet Red Bile Glucose (VRBG) medium; dishes were incubated at 37 °C for 24 h, according to BS ISO 21528-2:2004. The expression of the results was done according to ISO 7218:2007.

2.5. Statistical Analyses

The collected data were analyzed with R 4.0.5 software. An analysis of variance was realized to assess the significance of the variability of physico-chemical parameters. Poisson family models (Poisson and negative binomial models) were used for analyzing the variability of microbiological count data (Total viable count, yeast and molds, lactic acid bacteria and Enterobacteriaceae). Barplot test was performed as post-hoc test.

3. Results

3.1. Changes of the Physico-Chemical Characteristics of Mutchayan during Fermentation

Table 1 shows that, from 0 h to 120 h, the titratable acidity increases ($p < 0.00$) from 5.7 ± 0.1 to 6.8 ± 0.1 g of lactic acid for 100 g of the product (dry weight), while the pH did not change. The dry matter contents and the Brix value decreased significantly ($p < 0.00$) from 19.8 ± 0.0 to 16.7 ± 0.1 g/100 g and from 7.6 ± 0.11 °Bx to 5.0 ± 0.0 °Bx. The vitamin C content and the antioxidant capacity also varied significantly ($p < 0.05$) from 91.7 ± 1.1 to 69.8 ± 4.0 mg/100 dw and 657.1 ± 1.2 to 802.9 ± 48.9 mg VCEAC/100 g (dw) respectively.

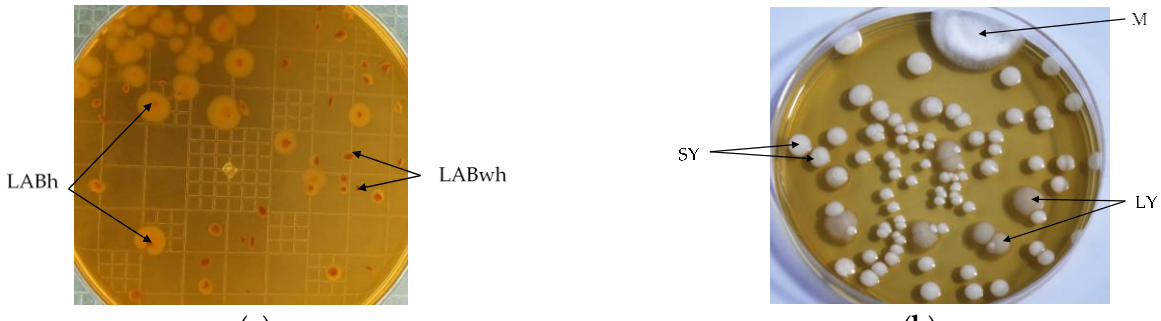
Table 1. Physico-chemical characteristics of sorghum flour, baobab pulp and *Mutchayan.*

Products Analyzed		pH	°Brix	TTA (LA g/100 g dw)	DM (g/100 g)	Vitamin C (mg/100 g dw)	Antioxidant Capacity (VCEAC mg/100 g dw)
Mutchayan samples of different lengths of fermentation	0 h	3.4 ± 0.0 ^{a}	7.3 ± 0.3 ^{a}	5.8 ± 0.1 $^{e.f}$	18.7 ± 0.1 ^{b}	91.7 ± 1.1 ^{a}	693.5 ± 49.0 $^{a.b}$
	6 h	3.4 ± 0.0 ^{a}	7.6 ± 0.1 ^{a}	5.7 ± 0.1 ^{f}	19.8 ± 0.0 ^{a}	84.7 ± 3.8 $^{a.b}$	699.2 ± 51.7 $^{a.b}$
	12 h	3.3 ± 0.0 ^{a}	7.5 ± 0.4 ^{a}	6.0 ± 0.0 $^{d.e}$	18.6 ± 0.2 ^{b}	79.1 ± 5.0 $^{a.b}$	677.7 ± 0.7 $^{a.b}$
	24 h	3.3 ± 0.0 ^{a}	7.4 ± 0.2 ^{a}	6.1 ± 0.0 $^{d.e}$	18.3 ± 0.0 ^{c}	69.8 ± 4.0 ^{b}	657.1 ± 1.2 ^{b}
	36 h	3.4 ± 0.0 ^{a}	6.8 ± 0.5 ^{a}	6.3 ± 0.0 $^{c.d}$	18.1 ± 0.0 ^{c}	78.8 ± 8.1 $^{a.b}$	713.8 ± 43.1 $^{a.b}$
	48 h	3.4 ± 0.0 ^{a}	6.0 ± 0.4 $^{a.b}$	6.5 ± 0.1 $^{a.b.c}$	17.5 ± 0.0 ^{d}	80.8 ± 1.1 $^{a.b}$	802.9 ± 48.9 ^{a}
	72 h	3.3 ± 0.0 ^{a}	6.2 ± 0.9 $^{a.b}$	6.8 ± 0.1 ^{a}	16.9 ± 0.0 ^{e}	76.6 ± 0.9 $^{a.b}$	766.1 ± 0.0 $^{a.b}$
	96 h	3.3 ± 0.0 ^{a}	5.0 ± 0.0 ^{b}	6.6 ± 0.1 $^{a.b}$	16.9 ± 0.1 ^{e}	79.3 ± 0.0 $^{a.b}$	751.5 ± 17.9 $^{a.b}$
	120 h	3.4 ± 0.1 ^{a}	5.1 ± 0.4 ^{b}	6.3 ± 0.1 $^{b.c.d}$	16.7 ± 0.1 ^{e}	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	-
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 with different letters in column are significantly different at 5% threshold. TTA: Total titratable acidity; DM: Dry matter.

3.2. Microbial Changes during Mutchayan Fermentation

Different types of microorganisms were observed during the fermentation. Among the lactic acid bacteria, two main types of colonies were observed: the colonies with a halo (LABh) and the others without halo (LABwh) (Figure 1); the same tendance was observed for yeasts: large yeast colonies (LY), and small yeast colonies (SY) which appeared on plate whiter and lighter (Figure 1).



(**a**) (**b**)

LABh: Lactic acid bacteria with halo; LABwh: Lactic acid bacteria without halo; SY: Small yeast; LY: Large yeast; M: Molds

Figure 1. (**a**) Lactic acid bacteria in *Mutchayan*; (**b**) Yeast and mold in *Mutchayan*.

At the beginning of the fermentation (0 h), the total viable count was estimated at about 3.5 ± 3.4 log_{10} cfu/g (Table 2), while the microbial activity of lactic acid bacteria, yeasts and Enterobacteriaceae was observed at 12 h of fermentation. Before 12 h, the dough temperature was decreasing from 57.8 ± 3.9 °C to 30.5 ± 0.0, and these microorganisms' loads were under 1 log_{10} cfu/g. While these microorganisms were absent, molds were revealed as dominant flora (at 0 h), with a population estimated at 2.7 log_{10} cfu/g; after an increase between 24 and 36 h, where their load reached a maximum estimated value of 6.9 log_{10} cfu/g, mold growth tended to be inhibited and the load decreasing below 5.6 log_{10} cfu/g at the end of the fermentation. The total viable count increased significantly ($p < 0.05$) from 12 to 36 h, reaching 7.8 ± 7.8 log_{10} cfu/g and keep constant till 120 h, with a dough temperature ranging from 28.0 to 32.3 °C (Table 2).

Table 2. Microorganisms count during *Mutchayan* fermentation.

Duration	TVC	YC	SYC	LYC	MC	LABC	LABwhC	LABhC	EC
0 h	h	<1	<1	<1	**2.7 ± 0.0**	<1	<1	<1	<1
6 h	3.6 ± 3.4 ^{c}	<1	<1	<1	3.0 ± 0.0	<1	<1	<1	<1
12 h	3.8 ± 2.9 ^{c}	3.1 ± 0.0 ^{d}	3.1 ± 0 ^{d}	<1	**2.7 ± 0.0**	2.7 ± 0.0 ^{d}	2.7 ± 0.0 ^{c}	<1	**1.7 ± 1.2** ^{b}
24 h	6.9 ± 7.0 ^{b}	7.0 ± 5.9 ^{c}	7.0 ± 0.0 ^{c}	**6.1 ± 0.0** ^{c}	<6.6	6.7 ± 6.7 ^{c}	6.5 ± 6.5 ^{b}	6.2 ± 6.3 ^{b}	<1
36 h	7.8 ± 7.8 ^{a}	7.4 ± 6.9 ^{b}	7.3 ± 6.7 ^{b,c}	6.4 ± 0.0 ^{b,c}	**6.9 ± 0.0**	7.4 ± 7.1 ^{b}	7.2 ± 6.9 ^{a,b}	7.0 ± 6.6 ^{a}	2.1 ± 0.9 ^{a}
48 h	7.7 ± 6.8 ^{a}	7.9 ± 7.4 ^{a}	7.8 ± 7.0 ^{a,b}	7.3 ± 0.0 ^{a}	<5.6	7.8 ± 6.4 ^{a,b}	7.8 ± 6.4 ^{a}	<5	<1
72 h	8.0 ± 7.6 ^{a}	8.0 ± 7.7 ^{a}	7.9 ± 7.7 ^{a}	**6.8 ± 0.0** ^{b,c}	<6.6	7.8 ± 7.6 ^{a,b}	7.5 ± 7.3 ^{a}	7.5 ± 7.3 ^{a}	
96 h	8.2 ± 7.8 ^{a}	7.9 ± 6.9 ^{a}	7.9 ± 6.1 ^{a,b}	7.1 ± 0.0 ^{a,b}	**6.6 ± 0.0**	8.1 ± 6.9 ^{a}	8.0 ± 7.1 ^{a}	7.2 ± 6.6 ^{a}	
120 h	8.2 ± 7.4 ^{a}	8.1 ± 6.1 ^{a}	8.0 ± 7.4 ^{a}	7.6 ± 0.0 ^{a}	<5.6	8.1 ± 6.9 ^{a}	8.0 ± 6.9 ^{a}	7.5 ± 7.2 ^{a}	

The mean values with different letters in column are significantly different at 5% threshold. The values in bold represented the estimated count of microorganisms; TVC: Total viable count; YC: Yeast Count; SYC: Small Yeast count; LYC: Large Yeast Count; MC: Molds count; LABC: Lactic Acid Bacteria Count; LABwhC: Lactic acid bacteria without halo count ; LABhC: Lactic acid bacteria with halo Count; EC: Enterobacteriaceae Count.

Yeast count increased significantly ($p < 0.05$) (Table 2) during the fermentation process, from less than 3.1 ± 0.0 log_{10} cfu/g after 12 h to 7.9 ± 7.4 log_{10} cfu/g after 48 h of fermentation; only small yeasts colonies were detected at 12 h of fermentation. A significant increase of yeast population was observed from 12 to 24 h, for small yeast colonies and for large yeast colonies as well. Yeast population stayed statistically the same after 48 h of the fermentation; the same trends were observed for small yeasts count and large yeasts count which were respectively 7.8 ± 7.0 log_{10} cfu/g and 7.3 ± 0.0 log_{10} cfu/g.

Similarly to yeasts, lactic acid bacteria (LAB) count rapid increased between 12 and 36 h from 2.7 ± 0.0 to 7.4 ± 7.1 log_{10} cfu/g; after 48 h, the lactic acid bacteria count did not change significantly and stayed at 7.8 ± 6.4 log_{10} cfu/g (Table 2). Both lactic acid bacteria with and without halo presented similar growth tendencies.

The enterobacteriaceae count increased from <1 to 1.7 ± 1.15 log_{10} cfu/g between 6 and 12 h, and decreased from 1.7 ± 1.15 to <1 log_{10} cfu/g between 12 and 24 h. After 36 h of fermentation, similar observations were made; the enterobacteriaceae load increased from <1 to 2.06 ± 0.85 log_{10} cfu/g, and later decreased to <1 log_{10} cfu/g. Enterobacteriaceae appear to not play an important role during the fermentation of *Mutchayan*.

4. Discussion

Yeasts and 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 on physico-chemical parameters till 24 h. After 24 h of fermentation, the total flora and the lactic acid bacteria load became higher (about 6 log10 cfu/g), and that might allow rapid increase of lactate production from 36 to 120 h, leading to an increase of the titratable acidity content between the begin-

ning and the end of the fermentation. Despite the lactic acid production, the quantity produced during the fermentation was not sufficient to decrease the already acid pH of *Mutchayan* (pH = 3.4 at 0 h of fermentation) as observed for the fermentation of cereal-based products as *Mawè* [11] and *Gowé* [7], where the pH diminished from 6.1-6.2 to 3.5-3.6 [11], almost the pH of *Mutchayan* at 0 h of fermentation.

The absence 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). But the presence of the molds at the same time is probably related to their ability to survive in critical conditions; some mold species can grow at low pH values (pH = 3.2) and low water activity [12]. The inhibition of molds growth along the fermentation process could be linked to the LAB activity because it has been proven that some strains of *Lactobacillus* sp. have a strong inhibitory capacity of fungal growth; that is the case of *Lactobacillus plantarum* strain ITEM 17215 which inhibit the growth of mycotoxigenic fungi as *Aspergillus flavus* [13,14].

As the fermentation duration increased, yeasts and lactic acid bacteria counts became higher (6–8 $\log_{10}$ cfu/g) than at the beginning, inducing a significant increase of antioxidant capacity, associated to the activity of lactic acid bacteria without halo which should be deeply studied for their relation with the increasing of antioxidant capacity. 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, as Kenyan *Busaa*, *Kaffir* beer, Nigerian *Ogi*, *Pito*, *Sekete* and *Busa* [15].

The occurrence of Enterobacteriaceae in *Mutchayan*, which was observed in other products, e.g., in *Gowé* [16], in *Calugi*, [17], could be linked to unmastered surrounding conditions and the fermenter (plastic bucket) which was not tightly covered. During *Mutchayan* fermentation, the decrease of Enterobacteriaceae load could be due to the low pH values or the increase of the lactic acid bacteria activity, since it was reported that LAB produce bacteriocins, effective to control their growth [18,19].

5. Conclusions

The fermentation of *Mutchayan* is characterized by the domination of molds during the 12 first hours. Their growth was inhibited by the development of yeast and lactic acid bacteria which dominated the fermentation. Along this microbial changes, a decrease of Brix value, dry matter, and an increase of lactic acid and antioxidant capacity were observed with the increasing of duration. Further investigations are needed for identifying the different microbial species and their specific role in the fermentation of *Mutchayan.*

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