EVALUATION OF THE MICROBIOLOGICAL QUALITY OF *TCHAPALO* PROCESS PRODUCTS, AN IVOIRIAN TRADITIONAL BEVERAGE

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ABSTRACT

Tchapalo and sweet wort are two traditional beverages made from cereals and prized by the population. However, their processing takes place most often in deplorable hygienic conditions. The aim of this study was to evaluate the microbiological quality of *tchapalo* process products to establish microbiological criteria for the *tchapalo* and sweet wort. Samples of products from *tchapalo* processing were collected from three local areas in Abidjan district. Then pH was determined. Isolation and enumeration of spoilage and pathogenic bacteria were also investigated. Results showed that the heat sweet wort did not contain any microorganism. However, in the cold sweet wort, total aerobic mesophilic bacteria were predominant with a highest load at Abobo ($2.4 \times 10^5 \pm 2.08 \times 10^5$ cfu.ml^{-1}). In all *tchapalo* samples, total coliforms and thermotolerant coliforms were absent. In *tchapalo* collected at Yopougon, counts of anaerobic sulfite-reducing bacteria and *S. aureus* were the highest. Their counts were $5.5 \times 10^2 \pm 2.1 \times 10^1$ cfu.ml^{-1} and $2.0 \times 10^2 \pm 1.4 \times 10^1$ cfu.ml^{-1} respectively. The evaluation of the hygienic quality of heat sweet wort, cold sweet wort and *tchapalo* showed that the microbiological quality of the heat sweet wort was satisfactory; but those of cold sweet wort and *tchapalo* were not acceptable. It is necessary to apply good hygienic practices during *tchapalo* process in order to ensure the microbiological quality of *tchapalo* and sweet wort.

Key words : *Tchapalo* process, Traditional beverages, Microbiological quality, Safety, Fermentations.

RESUME

EVALUATION DE LA QUALITE MICROBIOLOGIQUE DES PRODUITS ISSUS DE LA PRODUCTION DU* TCHAPALO*, UNE BOISSON TRADITIONNELLE IVOIRIENNE

Le tchapalo *et le moût sucré sont deux boissons traditionnelles à base de céréales prisées par la population. Cependant, leur production se déroule le plus souvent dans des conditions hygiéniques déplorables. L'objectif de cette étude était d'évaluer la qualité microbiologique des produits issus de la production du* tchapalo*, afin d'établir des critères microbiologiques pour le* tchapalo *et le moût sucré. Durant la production du* tchapalo*, des échantillons ont été prélevés dans trois communes du district d'Abidjan. Ensuite, le pH a été déterminé. Les bactéries pathogènes et d'altération alimentaire ont été isolées et dénombrées. Les résultats ont montré que le moût sucré fraichement préparé était exempt de micro-organisme. Toutefois, dans le moût sucré refroidi, les bactéries aérobies mésophiles totales étaient prédominantes avec une charge plus élevée à Abobo ($2{,}4 \times 10^5 \pm 2{,}08 \times 10^5$ cfu.ml^{-1}). Dans tous les échantillons de* tchapalo*, les coliformes totaux et les coliformes thermotolérants étaient absents. Dans le* tchapalo *de Yopougon, les charges des bactéries anaérobies sulfito-réducteurs et de* S. aureus *étaient les plus élevées ($5{,}5 \times 10^2 \pm 2{,}1 \times 10^1$ ufc ml^{-1} et $2{,}0 \times 10^2 \pm 1.4 \times 10^1$ ufc ml^{-1} respectivement). L'évaluation de la qualité hygiénique de moût sucré fraichement préparé, du moût sucré refroidi et de* tchapalo *a montré que la qualité microbiologique du moût sucré fraichement préparé est satisfaisante ; mais celles du moût*

sucré froid et du tchapalo *n'étaient pas acceptables. Il est nécessaire d'appliquer les bonnes pratiques d'hygiène lors de la production du* tchapalo *pour assurer la qualité microbiologique du* tchapalo *et du moût sucré ainsi que la sécurité du consommateur.*

Mots-clés : *Production du* tchapalo*, Boissons traditionnelles, Qualité microbiologique, Sécurité alimentaire, Fermentations.*

INTRODUCTION

Fermentation is one of the oldest technologies used to enhance taste, aroma, shelf-life, texture, nutritional value and other attractive properties of food. It is carried out in many parts of the world, with regional differences depending on the availability of raw materials, consumption habits and time to carry out processes (Aka *et al*., 2014). In Africa, fermentation is used to produce several food and beverages. Beverages play a very important role in the dietary pattern of people in African developing countries (Kouame *et al*., 2015). They have also a role in social functions such as marriage, naming and rain making ceremonies where they are served as inebriating beverages. These beverages take different names according to regions where they are produced; for example *dolo* in Burkina Faso and *pito* in Ghana (Sawadogo-Lingani *et al*., 2007), *bili-bili* in Chad (Maoura *et al*., 2006) and *tchapalo* in Côte d'Ivoire (N'Guessan *et al*., 2011; Amane *et al*., 2012).

Tchapalo is a traditional alcoholic beverage from sorghum grains. It is also produced from maize and millet. Its production is an old family tradition performed by women from the Northeast and northern part of Côte d'Ivoire. This production was first intended to family daily consumption. For these women, *tchapalo* production is today a real economic activity producing revenue throughout the whole country ; particularly in Abidjan where we have hundreds of production sites (Kouame *et al*., 2015 ; Amane *et al*., 2012). It thus develops a sorghum beer industry that sustains many families in Côte d'Ivoire and even in the sub region.

The brewing of *tchapalo* involves malting, drying, milling, mashing, souring (spontaneous fermentation), boiling, cooling and fermentation (spontaneous alcoholic fermentation). The spontaneous fermentation depends on environmental and climatic conditions and confers the souring taste and storage longevity. The alcoholic fermentation is usually initiated by dried yeast harvested from previous *tchapalo* (Djè *et al*., 2008). These fermentations are not controlled. Unfortunately, the production takes place most often in deplorable hygienic conditions with rudimentary equipment and laborious activities (Kouame *et al*., 2015). Drying of germinated sorghum grain is done always in the open air, along the tracks. Successive sweet worts are inoculated with the starter from previous fermentations, without knowing very well the real nature of this starter. The beers thus obtained have a short shelf-life storage (3 days) and their qualities varied from one production to another (Djè *et al.,* 2008; Amane *et al*., 2012; Kouame *et al*., 2015). It often occurs losses due to poor quality products which reduce the earnings of the brewers. To ensure food security of *tchapalo* and sweet wort, the establishment of the microbiological quality criteria is essential. This study aimed at increasing knowledge on the microbiological quality of products from different steps during *tchapalo* process in order to establish quality standards of the final products.

MATERIALS AND METHODS

TCHAPALO PROCESS

Tchapalo processing is described according to Aka *et al*. (2008a). Briefly, the process started by the malting of sorghum grain, sun-drying and milling to give malted sorghum flour. This flour was mixed out with water containing a sticky substance. The mixture obtained called mash was separated in supernatant and sediment. The sediment was precooked during 2 - 2 h 30 min; later mixed with the supernatant to give wort. The wort was left for a spontaneous lactic fermentation during the night to give after percolation the sour wort. This sour wort was cooked during 4-6 h to give sweet wort which was cooled and inoculated with dried yeast harvested from previous *tchapalo* for alcoholic fermentation during 9 -12 h. The product obtained after alcoholic fermentation is called *tchapalo*.

SAMPLING

Sampling was carried out on mash, cooked sediment, wort, sour wort, heat sweet wort freshly produced ie about 95 to 100°C, cold sweet wort at ambient temperature, traditional starter and *tchapalo* during each *tchapalo* processing. Samples were collected from three local areas ie Abobo, Attecoube and Yopougon in Abidjan district. They were selected because they are areas of mass production of *tchapalo*. They were collected in sterile bottles, labelled and then transported to the laboratory in a box containing a freezing pack. Four productions samples were collected from each area. A total of eighty-four samples were taken.

DETERMINATION OF PH

The pH was determined with a pH-meter (pH-meter P 107, CONSORT, Bio block, France) and two independent measurements were made on each sample.

ISOLATION AND ENUMERATION OF MICRO-ORGANISMS

10 ml of sample was homogenized in 90 ml sterile peptone water (pH 7.0) to obtain a 1:10 dilution. Further 10- fold dilutions were prepared from this and appropriate dilutions were spread in triplicate on different media. Total aerobic mesophilic bacteria were enumerated on plate count agar (AFNOR, NF V 08-051) and the plates were incubated at 30°C for 72 h. Total coliforms and thermotolerant coliforms were enumerated on violet red bile lactose agar (OXOID, ISO 4832 : 2006) and incubated at 30°C and at 44.5°C for 24h respectively. Growth of red colonies indicated the presence of coliforms. Positive plates were confirmed on Eosin methylene blue agar (Sigma-Aldrich, Leininger *et al*., 2001) at 37°C for 24h for *E. coli*. *Staphylococcus aureus* was enumerated on Baird Parker Agar (Sigma-Aldrich, NF EN ISO 6888-1). The plates were incubated at 37°C for 48h. The positive colonies were confirmed on mannitol salt agar (Sigma-Aldrich) and were further identifiedby Gram staining reaction, catalase test, DNase test, coagulase test with the rabbit plasma. Yeasts were enumerated on Sabouraud-chloramphenicol agar (AFNOR, NF ISO 7954) after 3 to 5 days of incubation at 25°C. Lactic acid bacteria were enumerated on Man Rogosa Sharpe Agar (AFNOR, NF ISO 15214). The plates were incubated at 30°C for 48 h under anaerobic conditions using anaerobic jar. Positive plates were identified by cultural and microscopic examination as well as by biochemical tests such as catalase and oxidase activities. Sulfite-reducing anaerobic spore counting was done on tryptone-sulfite-neomycin agar after appropriate dilutions were heat-treated at 80°C for 10 min in water bath (Norme NF T 90-415). Plates were incubated at 37°C for 24-48 h. *Salmonella* were analyzed by the procedure of the French standardization association (AFNOR, V 08 - 052).

STATISTICAL ANALYSIS

The data were analysed using one-way analysis of variance (ANOVA) (statistical, 99th edition). Duncan's multiple range test was used to compare the means when a significant variation was established by ANOVA at the significance level ($\alpha = 0.05$).

RESULTS

EVOLUTION OF PH DURING *TCHAPALO* PROCESS

The pH values varied significantly ($P < 0.05$) from 5.25 ± 0.08 in Yopougon mash to 5.84 ± 0.2 in Attecoube mash and 5.96 ± 0.6 in Abobo mash (Figure 1). This value remained relatively constant until wort and did not vary significantly ($P > 0.05$) between process in each area. After spontaneous fermentation, the pH decreased from 5.94 ± 0.24 in the Abobo wort to 3.96 ± 0.05 in the Abobo sour wort and varied significantly ($P < 0.05$) to Attecoube and Yopougon sour wort. The pH of Yopougon sour wort was the lowest (3.43 ± 0.12). The pH of other products from following operations (heat sweet wort, cold sweet wort and *tchapalo*) did not vary significantly ($P > 0.05$) between process and areas. However, pH of traditional starter was higher than that of *tchapalo* and it did not vary significantly ($P > 0.05$) between areas. The minimum pH value was observed in Yopougon traditional starter (pH 5.25 ± 0.04).

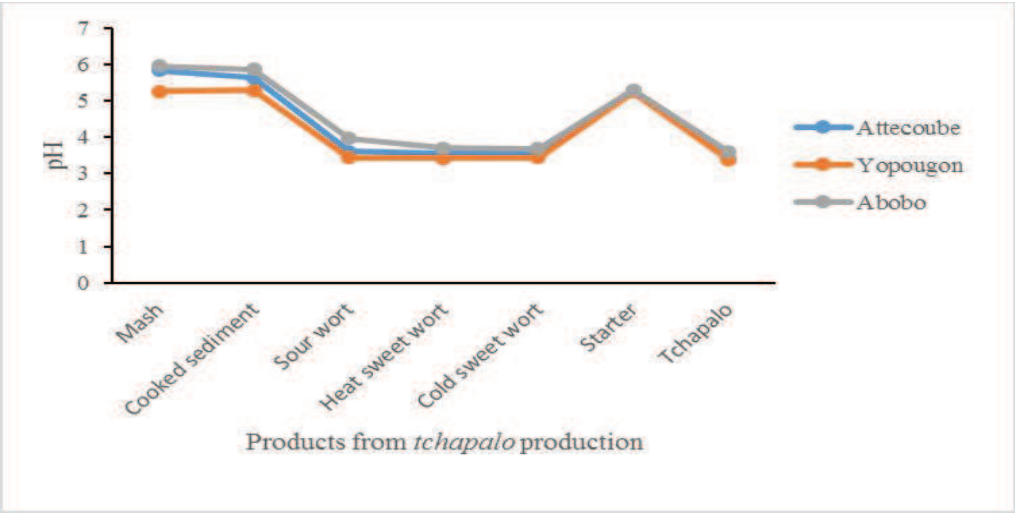

Figure 1 : pH variations during *tchapalo* process.

Variations du pH durant la production du Tchapalo.

PREVALENCE OF MICROORGANISMS DURING *TCHAPALO* PROCESS

Except *Salmonella* that was absent in all samples analysed, all other microorganisms (total aerobic mesophilic bacteria, lactic acid bacteria, yeasts, total coliforms, thermotolerant coliforms, *S. aureus* and spore of anaerobic sulfite-reducing bacteria) were present in all samples analysed with frequencies of occurrence varying from one microorganism to another according to the sample (Table 1). For example, all microorganisms were always present in the mash except *S. aureus* that was detected into 83.33 % of mash samples. Lactic acid bacteria and yeasts were only present in the 16.67 % cooked sediment samples. On the other hand, all microorganisms analysed were absent in the sweet wort freshly obtained. *Staphylococcus aureus* was found in 8.33 % and 41.67 % of cold sweet wort and *tchapalo* samples respectively ; 33.33 % of *tchapalo* samples contained anaerobic sulfite-reducing bacteria. Total coliforms and thermotolerant coliforms were s absents in *tchapalo* samples. But they were present in traditional starter samples.

Table 1 : Prevalence of microorganisms in different products obtained during *tchapalo* process.

Prévalence des microorganismes dans les différents produits obtenus lors de la production du tchapalo.

Products	Presence of microorganisms in products from *tchapalo* process (%)							
	TAMB	LAB	Yeast	TC	ThC	*S. a*	SRA	*Sal*
Mash	100	100	100	100	100	83.33	100	0
CS	58.33	16.67	16.67	0	0	0	100	0
Wort	100	100	100	66.67	66.67	66.67	91.67	0
SW	100	100	100	16.67	16.67	0	16.67	0
HSW	0	0	0	0	0	0	0	0
CSW	100	66.67	100	0	0	8.33	0	0
Tchapalo	100	66.67	100	0	0	41.67	33.33	0
Starter	100	91.67	100	66.67	58.33	75	83.33	0

TAMB : Total aerobic mesophilic bacteria, LAB : Lactic acid bacteria, TC : Total coliforms, ThC : Thermotolerant coliforms, SRA : Sulfite-reducing anaerobic spore, CS : Cooked sediment, SW : Sour wort, HSW : Heat sweet wort, CSW : Cold sweet wort, *Sal* : *Salmonella*

EVOLUTION OF MICROORGANISMS DURING *TCHAPALO* PROCESS

Before the spontaneous fermentation

Total aerobic mesophilic bacteria counts were high into all mash samples in the three areas. Counts of total coliforms were higher in Yopougon ($2.3 \times 10^6 \pm 1.1 \times 10^6$ cfu.ml^{-1}) and Abobo ($2.6 \times 10^6 \pm 1.7 \times 10^6$ cfu.ml^{-1}) mash samples than those of other microorganisms in the same areas (Tables 2 and 3). At Attecoube, lactic acid bacteria were predominant in all the samples ($1.5 \times 10^7 \pm 2.1 \times 10^6$ cfu.ml^{-1}, Table 4). On the other hand, in cooked sediments, total coliforms, thermotolerant coliforms and *S. aureus* were not detected except the spores of anaerobic sulfite-reducing bacteria. Their counts were respectively $4.0 \times 10^1 \pm 0.1 \times 10^1$, $6.0 \times 10^1 \pm 0.1 \times 10^1$ and $1.1 \times 10^2 \pm 1.0 \times 10^1$ cfu.ml^{-1}in Attecoube, Abobo and Yopougon cooked sediments. There was no significant difference ($P > 0.05$) between these counts. The loads of all microorganisms obtained in wort were very low compared to those obtained in the mash.

Table 2 : Counts of micr-organisms found into products from *tchapalo* process from Yopougon (cfu.ml^{-1})

Dénombrement des microorganismes présents dans les différents produits obtenus lors de la production du tchapalo à Yopougon (ufc.ml^{-1}).

Products Microorganisms	Mash	CS	Wort	Sour wort	HSW	CSW	*Tchapalo*	Starter
TAMB	$9.67x10^{6}$ $\pm 6.6x10^{5e}$	$1.0x10^{2}$ $\pm 9.4x10^{1b}$	$2.28x10^{3}$ $\pm 2.8x10^{2c}$	$3.7x110^{7}$ $\pm 3.6x10^{7e}$	$<1^{a}$	$1.04x10^{5}$ $\pm 1.07x10^{4d}$	$9.2x10^{8}\pm$ $9.7x10^{7f}$	$1.4x10^{11}$ $\pm 1.0x10^{11g}$
LAB	$1.12x10^{6}$ $\pm 8.3x10^{5d}$	$8.8x10^{1}$ $\pm 1.3x10^{1ab}$	$5.39x10^{2}$ $\pm 6.3x10^{1c}$	$4.5x10^{7}$ $\pm 6.9x10^{6e}$	$<1^{a}$	$0.78x10^{1}$ $\pm 0.6x10^{1ab}$	$1.8x10^{1}$ $\pm 2.1x10^{1ab}$	$2.9x10^{1}$ $\pm 0.9x10^{1bc}$
Yeast	$6.22x10^{5}$ $\pm 2.9x10^{5d}$	$1.4x10^{1}$ $\pm 2.1x10^{1a}$	$3.0x10^{2}$ $\pm 3.6x10^{1b}$	$1.4x10^{5}$ $\pm 1.3x10^{5d}$	$<1^{a}$	$9.6x10^{3}$ $\pm 1.1x10^{3c}$	$7.8x10^{8}$ $\pm 9.8x10^{7e}$	$2.7x10^{10}$ $\pm 3.4x10^{9f}$
Total coliforms	$2.3x10^{6}$ $\pm 1.1x10^{6c}$	$<1^{a}$	$9.05x10^{1}$ $\pm 3.04x10^{1b}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$1.1x10^{2}$ $\pm 8.1x10^{1b}$
ThC	$8.5x10^{5}$ $\pm 5.1x10^{5c}$	$<1^{a}$	$6.3x10^{1}$ $\pm 1.1x10^{1b}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$3.6x10^{1}$ $\pm 0.7x10^{1ab}$
S. aureus	$1.95x10^{2}$ $\pm 0.3x10^{1bc}$	$<1^{a}$	$1.25x10^{2}$ $\pm 1.3x10^{1ab}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$2.0x10^{2}$ $\pm 1.4x10^{1ab}$	$9.7x10^{2}$ $\pm 1.1x10^{1c}$
SRA	$3.18x10^{3}$ $\pm 2.7x10^{2d}$	$1.1x10^{2}$ $\pm 1.03x10^{1bc}$	$2.5x10^{2}$ $\pm 1.8x10^{1bc}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$5.5x10^{2}$ $\pm 2.1x10^{1b}$	$4.1x10^{2}$ $\pm 2.5x10^{1cd}$

TAMB : Total aerobic mesophilic bacteria, LAB : Lactic acid bacteria, ThC : Thermotolerant coliforms, SRA : Sulfite-reducing anaerobic spore, CS : Cooked sediment, HSW : Heat sweet wort, CSW : Cold sweet wort ; the averages having the same letter in the lines mean that there is no significant difference (P > 0.05)

Table 3 : Counts of microorganisms found into products from *tchapalo* process from Abobo (cfu.ml^{-1}).

Dénombrement des microorganismes présents dans les différents produits obtenus lors de la production du tchapalo à Abobo (ufc.ml^{-1}).

Products Microorganisms	Mash	CS	Wort	Sour wort	HSW	CSW	*Tchapalo*	Starter
TAMB	$1.3x10^{6}$ $\pm1.9x10^{5d}$	$4.0x10^{1}$ $\pm3.5x10^{1b}$	$1.5x10^{3}$ $\pm7.5x10^{2c}$	$2.0x10^{6}$ $\pm3.1x10^{5e}$	$<1^{a}$	$2.4x10^{5}$ $\pm2.1x10^{5de}$	$5.1x10^{8}$ $\pm5.4x10^{7f}$	$4.4x10^{8}$ $\pm2.0x10^{8f}$
LAB	$2.2x10^{5}$ $\pm2.8x10^{4d}$	$<1^{a}$	$1.9x10^{3}$ $\pm7.4x10^{2c}$	$7.7x10^{6}$ $\pm5.4x10^{6e}$	$<1^{a}$	$1.7x10^{2}$ $\pm1.2x10^{2b}$	$2.0x10^{3}$ $\pm2.1x10^{2c}$	$2.8x10^{3}$ $\pm2.5x10^{3c}$
Yeast	$3.5x10^{4}$ $\pm1.7x10^{4C}$	$<1^{a}$	$1.2x10^{3}$ $\pm7.5x10^{2b}$	$3.3x10^{4}$ $\pm3.1x10^{4C}$	$<1^{a}$	$2.1x10^{3}$ $\pm1.8x10^{3b}$	$5.6x10^{8}$ $\pm4.2x10^{8e}$	$6.1x10^{7}$ $\pm5.6x10^{7d}$
Total coliforms	$4.4x10^{5}$ $\pm3.4x10^{5c}$	$<1^{a}$	$3.6x10^{2}$ $\pm5.1x10^{1b}$	$3.7x10^{1}$ $\pm1.7x10^{1ab}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$2.4x10^{3}$ $\pm5.9x10^{2b}$
ThC	$5.9x10^{4}$ $\pm7.4x10^{3c}$	$<1^{a}$	$3.3x10^{1}$ $\pm4.0x10^{1ab}$	$1.2x10^{1}$ $\pm0.5x10^{1ab}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$2.3x10^{2}$ $\pm1.4x10^{2b}$
S. aureus	$2.1x10^{2}$ $\pm0.2x10^{1b}$	$<1^{a}$	$2.7x10^{2}$ $\pm1.5x10^{1b}$	$<1^{a}$	$<1^{a}$	$1.6x10^{2}$ $\pm0.1x10^{1ab}$	$1.0x10^{3}$ $\pm0.1x10^{1ab}$	$8.5x10^{2}$ $\pm7.1x10^{1ab}$
SRA	$1.9x10^{3}$ $\pm1.2x10^{2c}$	$6.0x10^{1}$ $\pm0.1x10^{1ab}$	$2.3x10^{2}$ $\pm1.5x10^{1b}$	$1.1x10^{2}$ $\pm0.5x10^{1ab}$	$<1^{a}$	$<1^{a}$	$1.0x10^{2}$ $\pm0.5x10^{1ab}$	$3.5x10^{2}$ $\pm3.4x10^{1b}$

TAMB : Total aerobic mesophilic bacteria, LAB : Lactic acid bacteria, ThC : Thermotolerant coliforms, SRA : Sulfite-reducing anaerobic spore, CS: Cooked sediment, HSW : Heat sweet wort, CSW : Cold sweet wort ; the averages having the same letter in the lines mean that there is no significant difference (P > 0.05)

Table 4 : Counts of microorganisms found into products from *tchapalo* process from Attecoube (cfu.ml^{-1}).

Dénombrement des microorganismes présents dans les différents produits obtenus lors de la production du tchapalo à Attecoube (ufc.ml^{-1}).

Products Microorganisms	Mash	CS	Wort	Sour wort	HSW	CSW	*Tchapalo*	Starter
TAMB	$7.5x10^{7}$ $\pm2.8x10^{6d}$	$1.3x10^{2}$ $\pm2.5x10^{1a}$	$1.5x10^{4}$ $\pm2.2x10^{3b}$	$3.2x10^{7}$ $\pm3.0x10^{7d}$	$<1^{a}$	$1.8x10^{5}$ $\pm1.4x10^{5c}$	$9.8x10^{8}$ $\pm1.4x10^{8e}$	$2.4x10^{10}$ $\pm1.2x10^{10f}$
LAB	$1.5x10^{7}$ $\pm2.1x10^{6d}$	$<1^{a}$	$1.4x10^{4}$ $\pm2.3x10^{4c}$	$3.6x10^{7}$ $\pm3.7x10^{6d}$	$<1^{a}$	$3.2x10^{2}$ $\pm5.9x10^{1ab}$	$1.6x10^{2}$ $\pm2.2x10^{1ab}$	$1.3x10^{3}$ $\pm1.4x10^{2b}$
Yeast	$3.9x10^{6}$ $\pm3.4x10^{6d}$	$<1^{a}$	$1.1x10^{4}$ $\pm1.9x10^{4b}$	$2.6x10^{5}$ $\pm2.8x10^{4d}$	$<1^{a}$	$1.3x10^{4}$ $\pm8.9x10^{3c}$	$9.3x10^{8}$ $\pm1.02x10^{8e}$	$1.7x10^{10}$ $\pm1.8x10^{9f}$
Total coliforms	$5.2x10^{6}$ $\pm3.5x10^{6c}$	$<1^{a}$	$8.3x10^{4}$ $\pm1.0x10^{4b}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$4.1x10^{3}$ $\pm1.5x10^{3b}$
ThC	$4.4x10^{6}$ $\pm5.0x10^{5c}$	$<1^{a}$	$2.0x10^{3}$ $\pm1.1x10^{3ab}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$2.2x10^{3}$ $\pm1.5x10^{3b}$
S. aureus	$7.5x10^{2}$ $\pm0.8x10^{1b}$	$<1^{a}$	$6.7x10^{1}$ $\pm0.2x10^{1b}$	$<1^{a}$	$<1^{a}$	$<1^{a}$	$1.0x10^{2}$ $\pm1.2x10^{1b}$	$1.7x10^{2}$ $\pm1.1x10^{1b}$
SRA	$4.2x10^{2}$ $\pm1.9x10^{1c}$	$4.0x10^{1}$ $\pm0.17x10^{1a}$	$1.1x10^{2}$ $\pm0.7x10^{1bc}$	$9.1x10^{1}$ $\pm0.1x10^{1ab}$	$<1^{a}$	$<1^{a}$	$1.0x10^{2}$ $\pm0.2x10^{1ab}$	$1.8x10^{2}$ $\pm0.5x10^{1bc}$

TAMB : Total aerobic mesophilic bacteria, LAB : Lactic acid bacteria, ThC : Thermotolerant coliforms, SRA : Sulfite-reducing anaerobic spore, CS : Cooked sediment, HSW : Heat sweet wort, CSW : Cold sweet wort ; the averages having the same letter in the lines mean that there is no significant difference (P > 0.05)

After the spontaneous fermentation

After spontaneous fermentation, counts of lactic acid bacteria in sour wort increased significantly ($P < 0.05$) compared to the other micro-organisms in all areas. There was also significant difference ($P < 0.05$) between lactic acid bacteria count of Abobo sour wort ($7.7 \times 10^6 \pm 5.4 \times 10^6$ cfu.ml^{-1}) and Attecoube sour wort ($3.6 \times 10^7 \pm 3.7 \times 10^6$ cfu.ml^{-1}) and Yopougon sour wort ($4.5 \times 10^7 \pm 6.9 \times 10^6$ cfu.ml^{-1}). Total coliforms, thermotolerant coliforms and *S. aureus* disappeared totally in all sour wort from Attecoube and Yopougon (Tables 2, 3 and 4). On the contrary counts of total coliforms and thermotolerant coliforms decreased in the Abobo sour wort to reach $3.7 \times 10^1 \pm 1.7 \times 10^1$ and $1.2 \times 10^1 \pm 0.5 \times 10^1$ cfu.ml^{-1} respectively. The anaerobic sulfite-reducing bacteria also decreased in the Attecoube and Abobo sour worts ($9.0 \times 10^1 \pm 0.1 \times 10^1$ cfu.ml^{-1} and $1.1 \times 10^2 \pm 0.5 \times 10^1$ cfu.ml^{-1} respectively) and they disappeared into Yopougon sour worts.

Alcoholic fermentation

The heat sweet wort did not contain any microorganism in all areas. However, in the cold sweet wort, total aerobic mesophilic bacteria were predominant with the highest load in Abobo ($2.4 \times 10^5 \pm 2.1 \times 10^5$ cfu.ml^{-1}). *Staphylococcus aureus* was only found in Abobo cold sweet wort (Table 3). Traditional starter was mainly composed of yeasts. The yeasts load varied significantly ($P < 0.05$) between starter from Abobo ($6.1 \times 10^7 \pm 5.6 \times 10^7$ cfu.ml^{-1}) and Attecoube ($1.7 \times 10^{10} \pm 1.8 \times 10^9$ cfu.ml^{-1}) and Yopougon starter ($2.7 \times 10^{10} \pm 3.4 \times 10^9$ cfu. ml^{-1}). But there was no significant difference ($P > 0.05$) between yeast counts in the starter from Attecoube and Yopougon . Total coliforms, thermotolerant coliforms *S. aureus* and anaerobic sulfite-reducing bacteria were also present in traditional starter from the three areas.

In *tchapalo*, only the loads of yeasts and the total aerobic mesophilic bacteria were increased significantly ($P < 0.05$) compared to other microorganisms in all areas. Their counts did not vary significantly ($P > 0.05$) between Attecoube and Yopougon (Tables 2 and 4). Total coliforms and thermotolerant coliforms were absent in all *tchapalo* from the three areas. The counts of Anaerobic sulfite-reducing bacteria and *S. aureus* were low and they did not vary significantly ($P > 0.05$) between areas. However, in Yopougon *tchapalo*, counts of anaerobic sulfite-reducing bacteria and *S. aureus* were the highest ($5.5 \times 10^2 \pm 2.1 \times 10^1$ cfu.ml^{-1}and $2.0 \times 10^2 \pm 1.4 \times 10^1$ cfu.ml^{-1} respectively).

relationship between micro-organisms and pH

THE HYGIENIC QUALITY OF HEAT SWEET WORT, COLD SWEET WORT AND *TCHAPALO*

The products consumed and marketed after the production of *tchapalo* are heat sweet wort, cold sweet wort and *tchapalo*. Microbiological criteria used for heat sweet wort and cold sweet wort in this study were those referred to standards and guidelines for the interpretation of analytical results in food microbiology of Quebec Government on infant cereal formulas (CECMA, 2009) because the sweet wort is intended for them too. These microbiological criteria were present in table 5. In light of these criteria, the microbiological quality of the heat sweet wort was satisfactory. So this product was acceptable for consumption. The mesophilic aerobic bacteria load in the cold sweet wort (1.7×10^5 cfu.ml^{-1}) was greater than the threshold value of the microbiological criteria The microbiological quality of the cold sweet wort was unsatisfactory and unsuitable for consumption. Microbiological criteria used for *tchapalo* in this study were those referred by Hanoi (2010) for alcoholic beverages and CFSFEHD (2011). In light of these criteria, the *tchapalo* quality was not acceptable; the mesophilic aerobic bacteria load (8.1×10^8 cfu.ml^{-1}) was greater than the threshold value of the microbiological criteria (< 10^5 cfu.ml^{-1}). The microbiological quality of the *tchapalo* was so unsatisfactory and therefore unsuitable for consumption.

Table 5 : Hygienic quality of heat sweet wort, cold sweet wort, *tchapalo* and microbiological criteria used.

Qualité hygiénique du moût sucré fraichement préparé, du moût sucré refroidi, du tchapalo et critères microbiologiques utilisés.

Products	TAMB	LAB	Yeast	TC	ThC	S. a	SRA	Sal	References
	cfu.ml^{-1}								
HSW	<1	<1	<1	<1	<1	<1	<1	Ab	This study
CSW	$1.7x10^5$	$1.6x10^2$	$8.3x10^3$	<1	<1	$1.6x10^2$	<1	Ab	This study
Tchapalo	$8.1x10^8$	$6.6x10^2$	$7.6x10^8$	<1	<1[a]	$4.8x10^2$	$3.2x10^2$	Ab	This study
Kunun-zaki	$6.3x10^6$	$54.2x10^6$	$20.5x10^6$	$5.2x10^6$	ND	P	ND	ND	Amusa and Odunbaku, 2009
Tchakpalo	$6.0x10^8$	$>5x10^4$	$>10x10^8$	P	P	$4x10^4$	ND	P	Baba-Moussa *et al.*, 2012
Ikigage	$33.5x10^6$	$35.3x10^4$	$10.1x10^6$	$32.3x10^3$	$21.9x10^3$	$16.0x10^3$	ND	ND	Lyumugabe *et al.*, 2010
Infant cereal formulas	$<1.0x10^4$	ND	ND	ND	10	$<1.0x10^2$	$<1.0x10^3$	Ab	CECMA, 2009
Bottle beer	10	ND	ND	ND	Ab	Ab	Ab	ND	Hanoi, 2010
Non-prepackaged beverages	$<10^5$	ND	ND	ND	100	100-<104	100-<104	N/A	CFSFEHD, 2011

TAMB : Total aerobic mesophilic bacteria, LAB : Lactic acid bacteria, TC : Total Coliforms, ThC : Thermotolerant coliforms, *S. a*: *Staphylococcus aureus*, SRA : Sulfite-reducing anaerobic spore, *Sa l*: *Salmonella*, HSW: Heat sweet wort, CSW: Cold sweet wort, Ab : Absence, ND : not determined ; P : presence ; N/A : not applicable

DISCUSSION

During *tchapalo* process, the microorganisms found in the mash derived from the raw material that is the sorghum malt. This raw material has been contaminated by the environment. Indeed, all analyzed microorganisms are encountered naturally in the environment or utensils or equipment used in the process of *tchapalo*. The mash may have been also contaminated by brewers themselves as stated Baba-Moussa *et al*. (2012). Besides the fermenting flora ie. yeasts and lactic acid bacteria present in the mash, total coliforms, thermotolerant coliforms (including *E. coli*), *S. aureus* and sulfite-reducing anaerobic spores that cause foodborne illness and gastroenteritis, were also found in the mash. Similar observations were made by other authors in cereal products (Lyumugabe *et al*., 2010 ; Ikpoh *et al*., 2013 ; Shankar and Usha, 2014).

The presence of these microorganisms in the sorghum malt is not surprising because often the process takes place in deplorable sanitary conditions (Aka *et al*., 2008b ; Kouame *et al*., 2015). A high load of total coliforms and thermotolerant coliforms can be a source of fecal contamination and therefore a lack of hygiene. However, according to Ballogou *et al*. (2011), it is possible to have good microbiological quality malt. Their study indicates that the controlled drying of sorghum malts, used for the *chakpalo* production, a traditional beverage from Benin, by using a shell drier improved the drying speed and the microbiological quality of the dried malts. Microorganisms were found in the supernatant and precooked sediment from the wort. Their low count is explained by the fact that the heat of precooked sediment had reduced enormously their abundance.

The wort was left for a spontaneous fermentation during the night to give the sour wort. Sour worts,that have the highest lactic acid bacteria loads, have a low pH also. High count in lactic acid bacteria was inversely proportional to the decrease in pH of the sour wort. Several authors have found that the process of African traditional beers has this spontaneous fermentation step (Maoura *et al*, 2006 ; Sawadogo-Lingani *et al*., 2007 ; AKa *et al*., 2008b ; N'Guessan *et al*., 2011 ; Kouame *et al*., 2015). This step is very important and obligatory because it leads to acidification and to extend the shelf-life of wort that later will give sweet wort and *tchapalo*. Spontaneous fermentation reduces the pH and prevents the growth of pathogens and spoilage microorganisms by the production of organic acid and hydrogen peroxide during the activities of lactic acid bacteria (AKa *et al*., 2008a ; Lyumugabe *et al*., 2010). The difference between European beers and traditional African beers is in the spontaneous fermentation step resulting in the obtaining of sour wort. This does not exist in European beers process (Lyumugabe *et al*., 2012).

Results of Yopougon sour wort are similar to other authors (Namugumya and Muyanja, 2009; Lyumugabe *et al*., 2010). The pH of the sour wort of this area was low and We observed that pathogens and spoilage microorganisms have disappeared. On the other hand, in the sour wort of Abobo, counts of pathogens and spoilage microorganisms were decreased but they persisted because probably the high pH values. This means that the acidification should be pushed to reach a pH of 3.5 in order to eliminate pathogens and spoilage microorganisms (Namugumya and Muyanja, 2009). The low pH value of Yopougon sour wort could be explained by the fact that the fermentation in this site is carried out over a long period. Indeed, the fermentation time is left to the appreciation of brewers. The fermentation ends only if the brewer considers the wort is sufficiently sour (Aka *et al*., 2008a ; Dje *et al*., 2008).

The heat sweet wort is free of germs and therefore sterile. It is the first product from the *tchapalo* process that is consumed by women, children and non-alcoholic consumers. The microbiological quality is satisfactory. However, during the cooling, this sweet wort was contaminated again by the utensils in which it is cooled or / and by the environment (Maoura *et al*., 2006) or /and by handling. The cooled sweet wort had a poor microbiological quality because the count of total aerobic mesophilic bacteria was high. Ikpoh *et al*. (2013) mention that the presence of *S. aureus* in the *Kunu*, a non-alcoholic beverage from cereal produced in Nigeria, even in small count could render a beverage unsuitable for human consumption. It is the same for the cooled sweet wort even if count of *S. aureus* is lower than microbiological criteria. Our results are similar to that found by Amusa and Odunbaku (2009) who found that laboratory *kunun zaki* drink harbored no coliform but it contained *S. aureus*. However, the same author found that, in the hawked *kunun-zaki*, counts of coliforms range from 2.2×10^6 cfu.ml^{-}

^{1} to 5.2 × 10^{6} cfu.ml^{-1} in Nigeria states analysis.

The traditional starter contained mostly yeasts that ensure the alcoholic fermentation to give the *tchapalo*. It also contained the spoilage and pathogens germs. However, *tchapalo* obtained does not contain total coliform and thermotolerant coliforms. The absence of these bacteria could be due to the presence of alcohol in the *tchapalo* and its acidity. According to Habamubgu *et al*. (2014), ethanol is an effective preservative when its concentration is sufficient. The fact that the *tchapalo* did not contain total coliforms and thermotolerant coliforms improves its microbiological quality. But the presence of pathogens such as S. *aureus* and sulfite-reducing anaerobic bacteria and the high load of total aerobic mesophilic bacteria in the *tchapalo* can deteriorate the same quality. All these microorganisms can eventually lead to sanitary risks to human health. Similar results have also been reported for other traditional fermented beverages (Namugumya and Muyanja, 2009 ; Lyumugabe *et al*., 2010 ; Baba-Moussa *et al*., 2012).

Lyumugabe *et al*. (2010) observed that marketed *Ikigage,* a traditional alcoholic beverage manufactured in Rwanda with malted sorghum, contained total aerobic mesophilic bacteria (33.5 × 10^{6} cfu.ml^{-1}), yeast (10.1 × 10^{6} cfu.ml^{-1}), lactic acid bacteria (35.3 × 10^{4} .ml^{-1}), moulds (4.1 × 10^{4} cfu.ml^{-1}), *E. coli* (21.9 × 10^{3} cfu.ml^{-1}), fecal streptococci (22.5 × 10^{3} cfu. ml^{-1}), *S aureus* (16.0 × 10^{3} cfu.ml^{-1}), total coliforms (32.3 × 10^{3} cfu.ml^{-1}). *Ikigage* is also characterized by absence of *Salmonella*. According to these authors, the microbiological analysis at the various stages of producing *ikigage* show that total coliforms and *S. aureus* disappear after fermentation. They concluded that these micro-organisms come from the post-fermentation process and therefore the final product presents a sanitary risk for consumers having a weakened immune system. The results of Baba-Moussa *et al*. (2012) on *tchakpalo*, a traditional beverage from Benin, revealed the presence of pathogenic microorganisms and worse hygienic indicators like the genus *Staphylococcus*, coliforms and *Salmonella*. The presence of these microorganisms constitutes a hazard for the consumers. Most of cereal traditional beverages have the same poor microbiological quality (Shankar and Usha, 2014). N'Guessan *et al*. (2011) identified *Saccharomyces cerevisiae* and *Candida tropicalis* as the yeasts species the most present in the *tchapalo*. Now the genus *Candida* is more and more considered as an emerging pathogen; hence the need to use a selected starter to produce the *tchapalo* is important.

The counts of total aerobic mesophilic bacteria in cold sweet wort and *tchapalo* were high because these beverages were not packaged and not sterilized after processed. Their microbiological quality was so unsatisfactory and therefore unsuitable for consumption. Obadina *et al*. (2010) adopted the HACCP system to produce *fufu*, an indigenous fermented cassava product produced in South-West Nigeria. They were able to reduce or even eliminate pathogenic microorganisms such as *E. coli*, *S. aureus* and *Salmonella* for assuring the safety of traditionally processed product.

CONCLUSION

Detailed knowledge of microbiological study of traditional *tchapalo* processing was a prerequisite for investigating ways to improve the microbiological qualities of the sweet wort and *tchapalo*. Spontaneous fermentation highly reduced or even eliminated the growth of pathogens and spoilage microorganisms. During *tchapalo* process, the cooking eliminated all microorganisms so the heat sweet wort was free of germs. However, during the cooling, this sweet wort was contaminated again. Hence, the cooled sweet wort had a poor microbiological quality. The traditional starter that is added to the cooled sweet wort to produce the *tchapalo* contained mostly yeasts but also often spoilage and pathogens bacteria. Although *tchapalo* obtained did not contain total coliforms, thermotolerant coliforms and *Salmonella*, pathogens such as *S. aureus* and sulfite-reducing anaerobic bacteria were a few times present. It contained also high load of total aerobic mesophilic bacteria. High counts of these microorganisms can eventually lead to sanitary risks for human health if corrective actions are not implemented. It is possible to obtain a good microbiological quality of the sweet wort and *tchapalo* by application of good manufacturing practice and good hygienic practices during *tchapalo* process to assure the safety of consumers. Therefore, starter cultures obtained with predominant lactic acid bacteria species and yeasts species would be selected

and used to produce sweet wort and *tchapalo*. This study will allow us also to establish our own microbiological criteria for the quality of traditional beverages.

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REFERENCES

Aka S., Camara F., Nanga Y.Z. Loukou Y.G., Dje K.M. 2008a. Evaluation of organic acids and sugars contents during the production of « *tchapalo* », a traditional sorghum beer in Côte d'Ivoire. *Journal of Food Technology* 6 (5) : 189 - 195.

Aka S., Djeni N.T., N'guessan K.F., Yao K.C., Dje K.M. 2008b. Variabilité des propriétés physico-chimiques et dénombrement de la flore fermentaire du « *tchapalo* », une bière traditionnelle de sorgho en Côte d'Ivoire. *Afrique Science* 4 (2) : 274 - 286.

Aka S., Konan G., Fokou G., Dje K.M., Bonfoh B.2014. Review on African traditional cereal beverages *American Journal of Research Communication* 2 (5) : 103 - 153.

Amané D.N., Kouamé K.B., Kouamé C., Assidjo E.N. 2012. Optimisation du procédé de fabrication du « *tchapalo* », bière traditionnelleivoirienne par le plan factoriel fractionné. *Afrique Science* 8 (3) : 69 - 81.

Amusa N.A., Odunbaku O.A. 2009. Microbiological and nutritional quality of « hawked kunun » (a sorghum based non-alcoholic beverag) widely consumed in Nigeria. *Pakistan Journal of Nutrition* 8 (1) : 20 - 25.

Baba-Moussa F., Bankole, S.H., Adeoti K., Ahouandjnou H., Gbenou J., Toukourou F., Sezan A., Kotchoni O.S., Baba-Moussa L. 2012. Study of microbiological quality of the fermented drink « *tchakpalo* » consumed in Benin roads.*International Research Journal of Microbiology* 3 (4) : 147 - 152.

Ballogou V.Y., Dossou J., de SouzaC.A. 2011. Controlled drying effect on the quality of sorghum malts used for the « *chakpalo* » production in Benin. *Food Nutrition Science* 2 : 156 - 161.

CECMA (Comité sur l'élaboration des critères microbiologiques dans les aliments), 2009. Lignes directrices et normes pour l'interprétation des résultats analytiques en microbiologie alimentaire. Centre québécois d'inspection des aliments et de santé animale, Quebec, Canada, 59p.https://www.mapaq.gouv.qc.ca/fr/Publications/recueil.pdf

CFSFEHD (Centre for Food Safety of the Food and Environmental Hygiene Department), 2011. Microbiological quality of non-prepackaged beverages mixed or topped with solid ingredients in Hong Kong. In : Government of the Hong Kong Special Administrative Region, ed. Risk Assessment Studies Report No. 46 : Microbiological Hazard Evaluation. Hong Kong, 25p.http://www.cfs.gov.hk/english/programme/programme_rafs/files/programme_rafs_fm_01_17_NPB.pdf

Djè M.K., N'Guessan K.F., Djeni T.N., Dadié T.A. 2008. Biochemical changes during alcoholic fermentation in the production of *tchapalo*, a traditional sorghum beer. *International Journal of Food Engineering* 4 (7) art. 2.

Habamubgu S.S.I., Kazadi M., Kafumba K.M. 2014. Evaluation chimique et microbiologique des boissons locales nouvellement introduites et produites par la population du Sud-Kivu : cas des groupements de Katanaet Bugorhe. *International Journal of Innovation and Applied Studies* 8 (2) : 736 - 742.

Hanoi 2010. National technical regulation of food safety for alcoholic beverages. *The Ministry of Health*, Vietnam, 12p.

Ikpoh I.S., Lennox J.A., Ekpo I.A., Agbo B.E., Henshaw E.E., Udoekong N.S. 2013. Microbial quality assessment of kunu beverage locally prepared and hawked in calabar, cross river state, Nigeria. *Global Journal of Biodiversity Science and Management* 3 (1) : 58 - 61.

Kouame K.B., Koko A.C., Masse D., Assidjo N.E. 2015. Batch fermentation process of sorghum wort modeling by artificial neural network. *European Scientific Journal* 11 (3) : 75 - 93.

Leininger D.J., Roberson J.R., Elvinger F. 2001. Use of eosin methylene blue agar to differentiate *Escherichia coli* from other Gram-negative mastitis pathogens. *Journal* of *Veterinary Diagnostic* Investigation 13 : 273 - 275.

Lyumugabe F., Kamaliza G., Bajyana E., Thonart P.H. 2010. Microbiological and physico-chemical characteristic of Rwandese traditional beer « *Ikigage* ». *African Journal of Biotechnology* 9 (27) : 4241 - 4246.

Lyumugabe F., Gros J., Nzungize J., Bajyana E., Thonart P.2012. Characteristics of African traditional beers brewed with sorghum malt: a review. *Biotechnologie Agronomie Société Environement* 16 (4) : 509 - 530.

Maoura N., Mbaiguinam M., Gaillardin C., Pourquie J. 2006. Suivi technique, analytique et microbiologique de la « *bili bili* », bière traditionnelle tchadienne. *Afrique Science* 20 (1) : 69 - 82.

N'Guessan K.F., Brou K., Noémie J., Casaregola S., Dje K.M. 2011. Identification of yeasts during alcoholic fermentation of « *tchapalo* », a traditional sorghum beer from Côte d'Ivoire. *Antonie van Leeuwenhoek* 99 (4) : 855 - 864.

Namugumya B.S., Muyanja C.M.B.K. 2009.Traditional processing, microbiological, physiochemical and sensory characteristics of « *kwete* », a ugandan fermented maize based beverage. *African Journal of Food Agriculture, Nutrition and Development* 9 (4) : 1046 - 1059.

Obadina A.O., Oyewole O.B., Sanni L.O., Tomlins K.I., Westby A. 2010. Improvement of the hygienic quality of wet « *fufu* » produced in South West Nigeria. *Food Control* 21 : 639 - 643.

Sawadogo-Lingani H., Lei V., Diawara B., Nielsen D.S., Møller P.L., Traoré, A.S., Jakobsen M. 2007. The biodiversity of predominant lactic acid bacteria in « *dolo* » and « pito » wort for the production of sorghum beer. *Journal of Applied Microbiology* 103 (4) : 765 - 777.

Shankar I.,Usha A. 2014. Assessment of the microbiological quality of « *koozh* », a fermented millet beverage. *African Journal of Microbiology Research* 8 (3) : 308 - 312.