EVALUATION OF THE MICROBIOLOGICAL QUALITY OF TCHAPALO PROCESS PRODUCTS, AN IVORIAN 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 × 10^5 ± 2.08 × 10^2 cfu.mL^-1). In all tchapalo samples, total coliforms and thermoduric 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 × 10^5 ± 2.1 × 10^4 cfu.mL^-1 and 2.0 × 10^5 ± 2.1 × 10^4 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

ÉVALUATION DE LA QUALITE MICROBIOLOGIQUE DES PRODUITS ISSUS DE LA PRODUCTION DU TCHAPALO, UNE BOISSON TRADITIONNELLE IVORIENNE

Le tchapalo et le moût sucré sont deux boissons traditionnelles à base de céréales prises 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 fraîchement préparé était exempt de micro-organisme. Toutefois, dans le moût sucré refroidi, les bactéries aérobiques mésothiles totales étaient prédominantes avec une charge plus élevée à Abobo (2.4 × 10^5 ± 2.08 × 10^2 cfu.mL^-1). Dans tous les échantillons de tchapalo, les coliformes totaux et les coliformes thermoduriques étaient absents. Dans le tchapalo de Yopougon, les charges des bactéries anaérobies sulfite-réducteurs et de S. aureus étaient les plus élevées (5.5 × 10^5 ± 2.1 × 10^4 cfu.mL^-1 et 2.0 × 10^5 ± 1.4 × 10^4 cfu.mL^-1 respectivement). L’évaluation de la qualité hygiénique de moût sucré fraîchement préparé, du moût sucré refroidi et de tchapalo a montré que la qualité microbiologique du moût sucré fraîchement 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 fermenta-
tion), boiling, cooling and fermentation
(spontaneous alcoholic fermentation). The
spontaneous fermentation depends on environ-
mental 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 9-12h. 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, Attécoube 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, CONSERT, 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 24 h 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 24 h 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 identified by 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. Sulphite-reducing anaerobic spore counting was done on tryptone-sulphite-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, 9th edition). Duncan’s multiple range test was used to compare the means when a significant variation was established by ANOVA at the significance level (α = 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 Attécoube 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 Attécoube 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).
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 absent 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.

<table>
<thead>
<tr>
<th>Products</th>
<th>Presence of microorganisms in products from Tchapalo process (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAMB</td>
</tr>
<tr>
<td>Mash</td>
<td>100</td>
</tr>
<tr>
<td>CS</td>
<td>58.33</td>
</tr>
<tr>
<td>Wort</td>
<td>100</td>
</tr>
<tr>
<td>SW</td>
<td>100</td>
</tr>
<tr>
<td>HSW</td>
<td>0</td>
</tr>
<tr>
<td>CSW</td>
<td>100</td>
</tr>
<tr>
<td>Tchapalo</td>
<td>100</td>
</tr>
<tr>
<td>Starter</td>
<td>100</td>
</tr>
</tbody>
</table>

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 x 10^6 ± 1.1 x 10^6 cfu.ml^-1) and Abobo (2.6 x 10^6 ± 1.7 x 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 x 10^7 ± 2.1 x 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 x 10^2 ± 0.1 x 10^2, 6.0 x 10^5 ± 0.1 x 10^5 and 1.1 x 10^6 ± 1.0 x 10^5 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 micro-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).*

<table>
<thead>
<tr>
<th>Products Microorganisms</th>
<th>Mash</th>
<th>CS</th>
<th>Wort</th>
<th>Sour Wort</th>
<th>HSW</th>
<th>CSW</th>
<th>Tchapalo</th>
<th>Starter</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAMB</td>
<td>9.67×10⁶</td>
<td>1.0×10²</td>
<td>2.28×10¹</td>
<td>3.7×10⁷</td>
<td>&lt;1⁎</td>
<td>1.04×10³</td>
<td>9.2×10⁹</td>
<td>1.4×10⁹</td>
</tr>
<tr>
<td></td>
<td>±6.6×10⁶a</td>
<td>±9.4×10⁶b</td>
<td>±2.8×10³c</td>
<td>±3.6×10⁶a</td>
<td>&lt;1⁎</td>
<td>±1.07×10⁶a</td>
<td>9.7×10⁷</td>
<td>±1.0×10⁷a</td>
</tr>
<tr>
<td>LAB</td>
<td>1.12×10⁶</td>
<td>8.8×10¹</td>
<td>5.3×10⁷</td>
<td>4.5×10⁷</td>
<td>&lt;1⁎</td>
<td>0.78×10¹</td>
<td>1.8×10¹</td>
<td>2.9×10¹</td>
</tr>
<tr>
<td></td>
<td>±8.3×10⁶a</td>
<td>±1.3×10³a</td>
<td>±6.3×10⁶a</td>
<td>±6.9×10⁶a</td>
<td>&lt;1⁎</td>
<td>±0.6×10³a</td>
<td>±2.1×10¹a</td>
<td>±0.9×10¹a</td>
</tr>
<tr>
<td>Yeast</td>
<td>6.22×10⁵</td>
<td>1.4×10¹</td>
<td>3×10⁷</td>
<td>1.4×10⁷</td>
<td>&lt;1⁎</td>
<td>9.6×10⁷</td>
<td>7.8×10⁷</td>
<td>2.7×10⁷</td>
</tr>
<tr>
<td></td>
<td>±2.9×10⁵a</td>
<td>±2.1×10⁴a</td>
<td>±3.6×10⁷a</td>
<td>±1.3×10⁷a</td>
<td>&lt;1⁎</td>
<td>±1.1×10³a</td>
<td>±9.8×10⁷a</td>
<td>±3.4×10⁷a</td>
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<td>Total coliforms</td>
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<td>9.05×10⁷</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>1.1×10⁸</td>
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<td>±1.1×10⁵a</td>
<td>±3.04×10⁴a</td>
<td>±3.04×10⁴a</td>
<td></td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>±8.1×10⁷a</td>
</tr>
<tr>
<td>ThC</td>
<td>8.5×10⁴</td>
<td>&lt;1⁎</td>
<td>6.3×10¹</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>3.6×10¹</td>
</tr>
<tr>
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<td>±1.1×10⁷a</td>
<td>±1.1×10⁷a</td>
<td></td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
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</tr>
<tr>
<td>S. aureus</td>
<td>1.95×10⁷</td>
<td>&lt;1⁎</td>
<td>1.25×10³</td>
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<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
<td>2.0×10²</td>
</tr>
<tr>
<td></td>
<td>±0.3×10³b</td>
<td>±1.3×10¹b</td>
<td>±1.3×10¹b</td>
<td></td>
<td>&lt;1⁎</td>
<td>±1.4×10⁷b</td>
<td>±1.1×10³b</td>
<td>9.7×10²</td>
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<tr>
<td>SRA</td>
<td>3.18×10³</td>
<td>1.1×10²</td>
<td>2.5×10²</td>
<td>&lt;1⁎</td>
<td>&lt;1⁎</td>
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<td>&lt;1⁎</td>
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<td>±1.03×10³b</td>
<td>±1.8×10³b</td>
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<td>&lt;1⁎</td>
<td>±2.1×10³b</td>
<td>±2.5×10³b</td>
<td>4.1×10²</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Products Microorganisms</th>
<th>Mash</th>
<th>CS</th>
<th>Wort</th>
<th>Sour Wort</th>
<th>HSW</th>
<th>CSW</th>
<th>Tchapalo</th>
<th>Starter</th>
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<tr>
<td>TAMB</td>
<td>1.3x10⁶</td>
<td>4.0x10⁵</td>
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<td>2.4x10⁵</td>
<td>5.1x10⁵</td>
<td>4.4x10⁵</td>
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<tr>
<td></td>
<td>±1.9x10⁵⁹</td>
<td>±3.5x10⁵⁸</td>
<td>±7.5x10⁶⁴</td>
<td>±3.1x10⁶⁶</td>
<td>±2.1x10⁶⁸</td>
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<td>±2.0x10⁶⁶</td>
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<td>±5.9x10⁷⁶</td>
<td>±5.9x10⁷⁶</td>
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<tr>
<td>ThC</td>
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<td>3.3x10⁷</td>
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<td>&lt;1⁹</td>
<td>&lt;1⁹</td>
<td>&lt;1⁹</td>
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<td>±4.0x10⁷⁰</td>
<td>±5.5x10⁷⁵</td>
<td>±1.4x10⁷⁶</td>
<td>±1.4x10⁷⁶</td>
<td>±1.4x10⁷⁶</td>
<td>±1.4x10⁷⁶</td>
<td>±1.4x10⁷⁶</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2.1x10⁷</td>
<td>&lt;1⁹</td>
<td>2.7x10⁷</td>
<td>&lt;1⁹</td>
<td>&lt;1⁹</td>
<td>1.6x10⁷</td>
<td>1.0x10⁷</td>
<td>8.5x10⁷</td>
</tr>
<tr>
<td></td>
<td>±0.2x10⁶⁶</td>
<td>±1.5x10⁷⁰</td>
<td>±0.1x10⁷⁵</td>
<td>±0.1x10⁷⁸</td>
<td>±0.1x10⁷⁸</td>
<td>±0.1x10⁷⁸</td>
<td>±0.1x10⁷⁸</td>
<td>±0.1x10⁷⁸</td>
</tr>
<tr>
<td>SRA</td>
<td>1.9x10⁷</td>
<td>6.0x10⁴</td>
<td>2.3x10⁷</td>
<td>1.1x10⁷</td>
<td>&lt;1⁹</td>
<td>1.0x10⁷</td>
<td>3.5x10⁷</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±1.2x10⁷⁶</td>
<td>±0.1x10⁷⁷</td>
<td>±1.5x10⁷⁰</td>
<td>±0.5x10⁷⁸</td>
<td>±3.4x10⁷⁶</td>
<td>±3.4x10⁷⁶</td>
<td>±3.4x10⁷⁶</td>
<td>±3.4x10⁷⁶</td>
</tr>
</tbody>
</table>

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).*

<table>
<thead>
<tr>
<th>Products Microorganisms</th>
<th>Mash</th>
<th>CS</th>
<th>Wort</th>
<th>Sour Wort</th>
<th>HSW</th>
<th>CSW</th>
<th>Tchapalo</th>
<th>Starter</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAMB</td>
<td>7.5x10⁷ ±2.8x10⁶&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3x10⁷ ±2.5x10⁶&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5x10⁷ ±2.2x10⁶&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2x10⁷ ±3.0x10⁶&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8x10⁷ ±1.4x10⁶&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.8x10⁶ ±1.4x10⁶&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4x10⁷ ±1.2x10⁶&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LAB</td>
<td>1.5x10⁷ ±2.1x10⁶&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4x10⁷ ±2.3x10⁶&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.6x10⁷ ±3.7x10⁶&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2x10⁷ ±5.9x10⁶&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6x10⁷ ±2.2x10⁶&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3x10⁷ ±1.4x10⁶&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yeast</td>
<td>3.9x10⁷ ±3.4x10⁶&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1x10⁷ ±1.9x10⁶&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6x10⁷ ±2.8x10⁶&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3x10⁷ ±8.9x10⁶&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.3x10⁶ ±1.02x10⁶&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.7x10⁷ ±1.8x10⁶&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>5.2x10⁷ ±3.5x10⁶&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.3x10⁷ ±1.0x10⁶&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1x10⁷ ±1.5x10⁶&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ThC</td>
<td>4.4x10⁹ ±5.0x10⁹&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0x10⁹ ±1.1x10⁹&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2x10⁹ ±1.5x10⁹&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S. aureus</td>
<td>7.5x10⁷ ±0.8x10⁹&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7x10⁷ ±0.2x10⁹&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0x10⁷ ±1.2x10⁹&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7x10⁷ ±1.1x10⁹&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SRA</td>
<td>4.2x10⁷ ±1.9x10⁷&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.0x10⁷ ±0.7x10⁷&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.1x10⁷ ±0.1x10⁷&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.1x10⁷ ±0.1x10⁷&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0x10⁷ ±0.2x10⁷&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.8x10⁷ ±0.5x10⁷&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

TAMB: Total aerobic mesophilic bacteria, LAB: Lactic acid bacteria, ThC: Thermotolerant coliforms, SRA: Sulfite-reducing anaerobic spore, CS: Cooked sediment, HSW: Heat sweet wat, CSW: Cold sweet wat; 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 other microorganisms in all areas. There was also significant difference (P < 0.05) between lactic acid bacteria count of Abobo sour wort (7.7 × 10⁶ ± 5.4 × 10⁵ cfu.ml⁻¹) and Attecoube sour wort (3.6 × 10⁷ ± 3.7 × 10⁶ cfu.ml⁻¹) and Yopougon sour wort (4.5 × 10⁷ ± 6.9 × 10⁶ 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 × 10¹ ± 1.7 × 10¹ and 1.2 × 10¹ ± 0.5 × 10¹ cfu.ml⁻¹ respectively. The anaerobic sulfite-reducing bacteria also decreased in the Attecoube and Abobo sour worts (9.0 × 10³ ± 0.1 × 10³ cfu.ml⁻¹ and 1.1 × 10³ ± 0.5 × 10¹ 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 × 10⁷ ± 2.1 × 10⁶ 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 × 10¹ ± 5.6 × 10⁰ cfu.ml⁻¹) and Attecoube (1.7 × 10¹³ ± 1.8 × 10⁶ cfu.ml⁻¹) and Yopougon starter (2.7 × 10¹² ± 3.4 × 10⁹ 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 × 10² ± 2.1 × 10¹ cfu.ml⁻¹ and 2.0 × 10² ± 1.4 × 10¹ cfu.ml⁻¹ respectively).

**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 (CECMR, 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 × 10⁶ 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 × 10⁶ cfu.ml⁻¹) was greater than the threshold value of the microbiological criteria (< 10⁶ cfu.ml⁻¹). The microbiological quality of the tchapalo was so unsatisfactory and therefore unsuitable for consumption.
<table>
<thead>
<tr>
<th>Products</th>
<th>TAMB</th>
<th>LAB</th>
<th>Yeast</th>
<th>TC</th>
<th>ThC</th>
<th>S. a</th>
<th>SRA</th>
<th>Sal</th>
<th>References</th>
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<tbody>
<tr>
<td></td>
<td>cfu ml$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>HSW</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
<td>Ab</td>
<td>This study</td>
</tr>
<tr>
<td>CSW</td>
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<td>&lt;1</td>
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<td>&lt;1</td>
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<td>This study</td>
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<td>6.6x10$^2$</td>
<td>7.6x10$^2$</td>
<td>&lt;1</td>
<td>&lt;1</td>
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<td>3.2x10$^2$</td>
<td>Ab</td>
<td>This study</td>
</tr>
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<td>54.2x10$^4$</td>
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<td>5.2x10$^4$</td>
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<td>P</td>
<td>ND</td>
<td>ND</td>
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<td>&gt;5x10$^3$</td>
<td>&gt;10x10$^3$</td>
<td>ND</td>
<td>P</td>
<td>4x10$^4$</td>
<td>ND</td>
<td>P</td>
<td>Baba-Moussa et al., 2012</td>
</tr>
<tr>
<td>Ikigage</td>
<td>33.5x10$^4$</td>
<td>35.3x10$^4$</td>
<td>10.1x10$^5$</td>
<td>32.3x10$^3$</td>
<td>21.9x10$^3$</td>
<td>16.0x10$^3$</td>
<td>ND</td>
<td>ND</td>
<td>Lyumugabe et al., 2010</td>
</tr>
<tr>
<td>Infant cereal</td>
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<td>ND</td>
<td>ND</td>
<td>10</td>
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<td>ND</td>
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<td>Ab</td>
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<td>Ab</td>
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<td>ND</td>
<td>ND</td>
<td>100</td>
<td>100-&lt;104</td>
<td>100-&lt;104</td>
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</table>

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: 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 i.e. 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
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 Habamubu 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 × 10^6 ml^-1), moulds (4.1 × 10^6 cfu.ml^-1), E. coli (21.9 × 10^6 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 unpolluted. 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 wont and tchapalo. This study will allow us also to establish our own microbiological criteria for the quality of traditional beverages.

ACKNOWLEDGMENTS

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REFERENCES


