Microbial contamination of the non-alcoholic beverage Gnamakoudji made from Zingiber officinale in Daloa, Côte d'Ivoire

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Street food has become unavoidable in the current scheme of restoration in urban Africa. This study aimed to study the microbial contamination of a refreshing artisanal drink, Gnamakoudji, made from Zingiber officinale sold in the city of Daloa (Côte d’Ivoire). Physicochemical tests showed that Gnamakoudji is an acidic drink (pH ≈ 3.83) and very sweet (0.2 gmL⁻¹). Microbiological analyses revealed a high level of contamination. For mesophilic aerobic germs, the CFU/mL load ranged from 6.5 × 10⁹ to 3.9 × 10¹⁰. The CFU/mL load for yeasts and molds ranged from 1.5 × 10⁶ to 1.4 × 10⁷. Total coliforms and enterobacteria ranged between 2.2 × 10⁴ to 1.7 × 10⁵ CFU/mL for the first and 1.7 × 10⁴ to 9.5x 10⁴ CFU/mL for the second. Worse, pathogenic bacterial species have been found in the Gnamakoudji of all neighborhoods. Gbokora and Grand-marche Gnamakoudji were contaminated with E. coli alone with 5x10⁵ and 4x10⁵ CFU/mL, respectively. That of the other neighborhoods contained both E. coli, S. aureus and Salmonella sp. The charges in E. coli and S. aureus oscillated between 4×10¹⁴ and 1.4×10⁵ for the first and 2.5×10⁴ to 5×10⁵ CFU/mL for the second. Salmonella sp was found in samples from the other four quarters (Commerce, Tazibouo, Labia and Soleil) with loads that ranged from 10⁵ to 1.4×10⁵ CFU/mL. These colony counts were unequally distributed and far exceeded the microbiological standards for juices and fruit drinks. The empirical manufacture of Gnamakoudji and the difficult sales conditions would increase the risk of contamination. The street Gnamakoudji is unfit for human consumption. Strategies to ensure the availability of a healthy Gnamakoudji must be put in place with government authorities, the private sector and consumers. This scientific data could help to develop codes of practice for the safety of Gnamakoudji in order to avoid diseases transmitted by street foods.

Key words: Microbial contamination, gnamakoudji, street food, Daloa, Côte d’Ivoire.

INTRODUCTION

In recent decades, street food has become an essential source of food in the major cities of developing countries. These foods are defined as ready-to-eat foods and beverages prepared and/or sold by street vendors or fixed vendors (FAO, 2014a). Rapid urbanization and the multiple constraints associated with it such as the distance between workplaces and home, poverty, the development of women’s activity, the breakdown of family
solidarity, and the emergence of new food styles would explain the expansion of this activity in Africa (Compaořè et al., 2008). The city of Daloa with 5,305 km² of area is the third most populous city of Côte d'Ivoire after Abidjan and Bouake. Its population increased from 163,537 in 1998 to a population of 245,350 at the last general census in 2014 (RGPH, 2014; Zah, 2015). Today, the population is estimated at more than 288,000 inhabitants.

With rampant urbanization, Daloa is also confronted with profound changes in lifestyles, work activities, family and social relations, which crystallize the problem of food security. Thus, this constantly growing population with the many administrative changes (commune, sub-prefecture, department and region, university town), has a clearly growing need for food, and street food has become a major source of food. Local craft drinks made from local fruits or vegetables are part of street foods. Of these, the drink Gnamakoudji (based on ginger (Zingiber officinale)) is very well known. This non-alcoholic, hot-tasting traditional drink is appreciated and abundantly well-known in many countries of the sub-region, as is the case in Côte d'Ivoire (Nandkangre et al., 2015; Mpondo et al., 2017). This drink, extracted from the rhizome, is a refreshing drink during weddings or baptisms. It is consumed alone as in family (Nandkangre et al., 2015). In Daloa, it is sold along roadsides, in markets and areas with high population densities such as schools and bus stations. Because of its cost accessible on all budgets, this refreshing drink is an important part of people’s eating habits. However, during processing, this drink can be contaminated by various microorganisms including pathogens.

In addition, street foods are known to be frequently associated with diseases and several microbiological analyses have revealed the presence of many pathogenic microorganisms with loads exceeding the standards (Barro et al., 2007; Chenouf et al., 2014; Mbadu et al., 2016). In addition, several outbreaks of disease have been attributed to beverages in various parts of the world and some infections have been reported in populations consuming these street foods (WHO, 2010; Mihajlovic et al., 2013; Kouassi et al., 2018). If the diet of this drink has proven positive aspects, the microbiological quality remains doubtful (Kouassi et al., 2012; Bayoï et al., 2014; Mbadu et al., 2016). Except, to our knowledge, no study on the quality of this soft drink sold in the city of Daloa has yet been the subject of scientific study. It is in this context that this study falls under the theme: Microbial contamination of a non-alcoholic soft drink sold in the streets of Daloa: the case of Gnamakoudji (Z. officinale). The overall objective of this study is to evaluate the microbial contamination of this non-alcoholic refreshing drink sold in the streets of Daloa. It also aims to diagnose the manufacturing process, looking for physicochemical parameters of this refreshing drink sold in the streets of Daloa. The information obtained can be used to educate producers and consumers about the quality and safety of this drink produced in the city of Daloa, or even throughout the country.

**MATERIALS AND METHODS**

The study material consists of the non-alcoholic drink called Gnamakoudji sold in the streets of Daloa. This drink is made from ginger (Z. officinale).

**Investigation for the diagnosis of the manufacturing process of Gnamakoudji**

Information on Gnamakoudji drink manufacturing processes was collected from 10 producers in the city of Daloa. The information concerned the different stages of the manufacture of the Gnamakoudji drink.

**Sampling**

Samples of Gnamakoudji were harvested in six (6) main districts of Daloa City (Commerce, Tazibouo, Gbokora, Labia, Grand marche and Soleil). In each neighborhood, three samples each consisting of three bottles (250 to 330 mL) were collected at the same point of sale. The various points of sale were either on the roadsides, in the markets or in areas with high population densities such as schools and bus stations. In these different outlets, drinks are exposed to room temperature. Samples once taken are stored in a cooler with dry ice and transported to the laboratory for analysis. These analyses were done at the Laboratory of Host-Microorganisms and Evolutions Interactions (LIHME) of University Jean Lorougnon Guede.

**Physicochemical characterization of Gnamakoudji**

**Determination of the pH**

The determination of the pH is carried out using a pH-meter (PHS-38W). A quantity of 150 mL of the sample was used for this purpose. Three (3) measurements from one sample of 150 mL were performed.

**Determination of Brix degree and amount of sugar in Gnamakoudji**

The degree of Brix was measured using a portable refractometer (ATAGO N-1α) measuring up to 30 Brix. Three measurements were performed per sample. For the determination of the amount of sugar in the beverages, the volume of beverage contained in the

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After a 1 hour rest on the bench at room temperature, the stock solution was decimally diluted in sterile Tryptone Salt medium up to $10^8$.

Inoculations and incubations

**Research of mesophilic aerobic germs, yeasts and molds, total coliforms, enterobacteria and Escherichia coli**

According to the prescriptions of the standards adopted, the pour plate method was applied. Thus, one milliliter (1 mL) of the diluted Gnamakoudji sample to be analyzed was aseptically transferred to a sterile Petri dish and mixed with 20 mL of the respective agar. After solidification, the dishes are inverted and incubated at temperatures as given in the respective standard. Three Petri dishes were inoculated per dilution. The characteristic colonies according to the different media are then counted taking into account the calculation standard (NF/ISO 7218: 2007).

**Research of Staphylococcus aureus**

The surface spreading method was used for the detection of S. aureus. It consisted of taking 0.1 mL of the stock solution or a dilution of the Gnamakoudji sample to be analyzed, using a sterile pipette, and transferring to a Petri dish containing the Baird Parker agar medium already poured and solidified. The dilution is spread on the agar using a spreader rake. These manipulations are all performed under aseptic conditions near the Bunsen burner flame. Petri dishes are then inverted and incubated at 37°C for 48 h. Two plates of petri were seeded by dilution. Black colonies with a clear halo (action of lecithin) and an opaque zone (action of lipase) are counted (15-150 characteristic colonies) taking into account the dilution.

**Highlighting Salmonella sp.**

It is done in three stages. Pre-enrichment is performed by incubating the stock solution at 37°C for 24 h. The enrichment consisted of taking 0.1 mL of the stock solution (pre-enriched) and transferred to a tube containing 10 mL of Vassiliadis Rappaport previously prepared and sterilized. After homogenization, the tube is incubated at 42°C for 24 h. Finally the isolation was carried out from the enrichment medium incubated on a solid selective medium: Hecktoen agar. A drop is taken using a Pasteur pipette and then seeded by streaks on the surface of the Hecktoen agar.

The dish is incubated at 37°C for 24 h, and sometimes even for 48 h, in the absence of characteristic colonies after the first incubation. On Hecktoen agar, the typical Salmonella colonies observed are green or blue with a black center.

**Enumeration**

The number of Colony Forming Units per milliliter of sample (CFU/mL) from the number of colonies obtained in the Petri dishes is carried out according to standard NF/ISO 7218: 2007

$$ N = \frac{\sum C_i}{(N_1 + 0.1N_2) d V} $$

$\sum C_i$: Sum of characteristic colonies counted on all retained Petri dishes;
$N_1$: Number of Petri dishes retained at the first dilution;
$N_2$: Number of Petri dishes retained at the second dilution;
$d$: Dilution rate corresponding to the first dilution;
$V$: Inoculated volume (mL);
$N$: Number of microorganisms (CFU/mL).

**Standards for assessing the microbiological quality of Gnamakoudji**

The microbiological quality assessment standards for Gnamakoudji are taken from the “Microbiological Criteria for Foodstuffs - Guidelines for Interpretation of 2015 of Luxembourg”.

**Statistical analyses**

The different parameters analyzed were then subjected to an analysis of variance (ANOVA) with the software Statistica, 99 Edition. For this purpose, a single-factor ANOVA and Duncan’s multi-extended tests were used. ANOVA was used to test, on the one hand, the variability between the different samples. As for Duncan’s test, he later made it possible to first locate the differences between the samples and then the differences between them. Statistical differences with P-values under 0.05 were considered significant.
Figure 1. Flow-Diagram of homemade Gnamakoudji.

Table 1. Physicochemical characteristics of Gnamakoudji sold in the main districts of Daloa.

<table>
<thead>
<tr>
<th>Variable</th>
<th>pH</th>
<th>Brix degree</th>
<th>Sugar level (g / mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commerce</td>
<td>3.90 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.53 ± 7.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.22 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tazibouo</td>
<td>3.84 ± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.73 ± 4.40&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.22 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gbokora</td>
<td>3.61 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.07 ± 1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Labia</td>
<td>3.68 ± 0.10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.93 ± 1.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Grand-marche</td>
<td>3.78 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.60 ± 0.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.20 ± 0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soleil</td>
<td>4.04 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.87 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.21 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>3.81 ± 0.12</td>
<td>18.62 ± 1.10</td>
<td>0.20±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

In columns, the values assigned to the same letter are not significantly different from (P> 0.05).

RESULTS

Evaluation of the manufacturing process of Gnamakoudji

All the information collected from the manufacturers, unit processing operations are essentially the same, with the exception of the rhizome peeling stage which is not carried out by some manufacturers. These operations are carried out on the same production site except the grinding phase which is carried out in another site (market, or grinding machine site). The essential operations are illustrated by the Gnamakoudji manufacturing diagram (Figure 1). However, no heat treatment is associated with the manufacture of this drink.

Physicochemical characteristics of Gnamakoudji

The physicochemical studies carried out on the various drinks made it possible to determine the Brix degree and the pH summarized in Table 1. Gnamakoudji is an acidic drink with an average pH of 3.81 ± 0.12. The Brix degree ranged between 16.93 and 19.73. The estimated
Gnamakoudji sugar level from Brix degrees and drink masses is about 0.20 g/mL.

Microbiological characteristics of Gnamakoudji

The microbiological studies carried out allowed the counting of microorganisms, the main weathering flora or flora evoking a lack of hygiene (yeasts, molds, mesophilic aerobic germs, total coliforms and enterobacteria) and pathogenic species in Gnamakoudji. Drinks from all sampled neighborhoods were heavily contaminated by these different floras. In addition, all flora charges (CFU/mL) were all well above the expected microbiological quality standards (norm NF in 2073/2005/CE). The CFU/mL load for yeasts and molds ranged from $1.5 \times 10^6$ to $1.4 \times 10^7$, whereas the standard predicts $10^5$ (Figure 2). The counts found in the Gnamakoudji neighborhood Soleil and Grand-marche were not statistically different ($p > 0.05$). For mesophilic aerobic germs, the CFU/mL load ranged from $6.5 \times 10^9$ to $3.9 \times 10^{10}$ while the standard indicates $10^6$ (Figure 3),
however, Gnamakoudji loads from Gbokora and Labia neighborhoods were not statistically different (p > 0.05). Total coliforms and enterobacteria ranged between $2.2 \times 10^4$ to $1.7 \times 10^5$ CFU/mL for the first and $1.7 \times 10^4$ to $9.5 \times 10^4$ CFU/mL for the second while the expected standard is $10^3$ CFU/mL (Figures 4 and 5). For total coliform loads in the Commerce and Labia neighborhoods, there was no statistically significant difference (p > 0.05); those in enterobacteria of the Soleil and Commerce districts also did not statistically show any difference (p > 0.05). In addition, the colony counts of the flora were also unevenly distributed. If Gnamakoudji from the Tazibouo district was more contaminated by mesophilic aerobic germs ($3.9 \times 10^{10}$ CFU/mL), the Gbokora District contained the highest yeast and mold load ($1.4 \times 10^7$ CFU/mL); Gnamakoudji in the Grand Marche District had the highest total coliform load ($1.7 \times 10^5$ CFU/mL) and the Soleil district, the highest level of enterobacteria ($9.5 \times 10^4$ CFU/mL).

Pathogenic bacteria species were detected all the drinks of different neighborhoods (Figure 6). Gbokora and Grand-marche Gnamakoudji were contaminated only with E. coli with $5 \times 10^5$ and $4 \times 10^5$ CFU/mL, respectively; loads higher than the standard of $10^5$. Other beverages from...
other neighborhoods contained both *Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp* with heavy loads exceeding the microbiological standards for juices and fruit drinks. The *E. coli* and *S. aureus* loads of all beverages ranged from $4 \times 10^4$ to $1.4 \times 10^5$ for the first and $2.5 \times 10^4$ to $5 \times 10^5$ CFU/mL for the second while the standard tolerated only $10^2$ for both species. Where the microbiological standard for *Salmonella sp* is a total absence in 25 mL of beverage, loads of $10^5$ to $1.4 \times 10^5$ CFU/mL were noted for Commerce, Tazibouo, Labia and Soleil samples.

The burden of pathogenic species varied from one district to another. Tazibouo's Gnamakoudji was more contaminated than those in other neighborhoods; it contained both the largest *E. coli* and *S. aureus* loads of $1.4 \times 10^5$ and $5 \times 10^4$ CFU/mL, respectively. Labia's had the largest load of *Salmonella sp* ($1.4 \times 10^5$ CFU/mL). The *E. coli* loads of Gnamakoudji from Commerce and Gbokora were not statistically different ($p>0.05$). Those in *S. aureus* from the Commerce and Labia neighborhoods were not as statistically different ($p>0.05$). *Salmonella sp* (CFU/mL) contamination of Gnamakoudji from Tazibouo, Labia and Soleil neighborhoods was not statistically different ($p>0.05$). The Gnamakoudji sold in the streets of Daloa is identified as a source of microbiological hazards, which would cause multiple diseases such as diarrhea, gastroenteritis, typhoid and paratyphoid fevers. Its consumption constitutes a real risk of infection or a source of food poisoning which can lead to a public health problem.

**DISCUSSION**

The flow diagram of artisanal production of Gnamakoudji compared with those presented in the work of Agassounon et al. (2011) and those of Ndiaye et al. (2015) on other close-up craft drinks were almost the same. Moreover, according to the work of Ndiaye et al. (2015), throughout West Africa, fruit juice and beverage product flow diagrams show the same unit operations with some differences in formulations. But, these empirical manufacturing methods do not guarantee the stability of the productions. However, the physicochemical stability and the microbiological quality of Gnamakoudji depend in particular on the raw material, the formulations and the various technological treatments including pasteurization, chemical preservatives and stabilized final pH. Physicochemistry showed that Gnamakoudji was an acidic drink. This acidity is due to the liberation of many organic acids (contained in the rhizomes of ginger in the juice (Ali et al., 2008; Park et al., 2008; Shimoda et al., 2010). The pH of this non-alcoholic beverage is satisfactory because according to CODEX-STAN 243-2003, the pH of fruit juices and beverages must be less than 4.5. With regard to the sugar content of Gnamakoudji, consumption approximately 330 mL (more than 50 g of sugar) would put the consumer at the limit of the tolerable amount recommended by the WHO (less than 50 g of sugar per day).

Over-consumption of non-alcoholic but very sweet Gnamakoudji could have adverse effects on health and lead to cardiovascular disease (Johnson et al., 2009, Yang et al., 2014), obesity (Sievenpiper et al., 2012; Wang et al., 2013), diabetes (Basu et al., 2013) and dental caries (Moynihan and Kelly, 2014). This 18% sugar content is lower than the levels that would limit microbial growth (between 65 and 67%) based on Boumendjel's work (2005). Microorganisms could therefore develop easily in said drinks despite relatively low pH. Indeed, at pH close to 4, germs are not
eliminated but have a slow growth. The microbiological study showed the contamination of *Gnamakoudji* by flora of deterioration and insalubrity resulting in a deficit of good production practices. Worse, pathogenic species are found there. This contamination is due, on the one hand to the process of transformation of the drink and on the other hand to the intrinsic characteristics of this drink.

According to the manufacturing process, ginger is often ground with the skin during the manufacture of *Gnamakoudji*. Like simple washing, no disinfection operation has been undertaken to neutralize the microorganisms of the soil on this skin. In addition, the hygienic state of mechanical grinders in community markets is never mastered. The multiplicity of products crushed by these machines could be considered as a cross-contamination site par excellence. Also, the operation of extraction of the juice would be carried out with bare hand. The hands would carry most of the germs of contamination, even faecal, when hygiene is not practiced. It should be noted, however, that the drink obtained was always packaged in jars already used or recycled; anything that does not guarantee their safety.

Moreover, according to the work of Bayoil et al. (2014), manufacturing processes greatly influence the microbiological quality of artisanal beverages. In a study on food packaging, recycled packaging is strongly discouraged as it may contaminate food (FAO, 2014b). Hygiene indicators such as *E. coli*, coliforms and enterobacteria have been detected at loads well in excess of standards. These germs could be responsible for serious food infections. Moreover, in the recent work of Kouassi et al. (2018), street food consumers in the same city reported gastroenteritis, diarrhea, vomiting and fevers from consuming foods such as fruit juices and drinks, or dairy products. These microorganisms indicative of a lack of hygiene have also been highlighted in other artisanal drinks by other authors such as Aawi (2000), Kruy et al. (2001) and Baba-Moussa et al. (2006).

In addition, the total absence of heat treatment throughout the manufacturing process would explain the very high load of indicator bacteria mesophilic aerobic germs in *Gnamakoudji*. The presence of these germs in artisanal beverages has also been reported by Mbadu et al. (2016). Poor conservation would favor the process of alteration of *Gnamakoudji*. Indeed, this drink is sold in coolers and jars without ice to maintain the temperature at 4°C, without omitting the phases of freezing and thawing. The breaking of the cold chain could cause the accelerated growth of certain microorganisms.

According to the work of Agassounon et al. (2006) and those of Folefack et al. (2008), in addition to manufacturing processes, the contamination of artisanal juices and beverages is also related to the socio-geographical environment of manufacture and / or sale of these juices or drinks. The fabrications were made in homes with uncontrolled environment. Under these conditions, these drinks would be subject to all kinds of contamination according to Koné (2014) in a study of the control of contamination of fresh drinks. The less acidic pH could help the growth of these germs. The presence of pathogenic bacterial species such as *S. aureus*, *E. coli* and *Salmonella* sp is a real danger for consumers of this drink. Apart from samples from Gbokora and Grand-marche districts, where the drinks were contaminated with only *E. coli*, the *Gnamakoudji* from the other quarters was contaminated by the three pathogenic species in combination: *S. aureus*, *E. coli* and *Salmonella*; anything that would not guarantee food security. Similar studies have reported not only common but also pathogenic germs in beverages (Agassounon et al., 2009; Amusa et al., 2009). The consumption of street *Gnamakoudji* poses a real risk of dietary infection for Daloa populations.

**Conclusion**

Street food has become unavoidable in the current development of urban catering, as is the case in Daloa. The physicochemical and microbiological analyses revealed that the consumption of the *Gnamakoudji* drink sold in the city of Daloa would pose a risk to the health of the consumer. High loads of coliforms, enterobacteria and mesophilic aerobic germs have been discovered. Pathogenic bacterial species such as *E. coli*, *S. aureus* and *Salmonella* sp even in high numbers have been detected in street *Gnamakoudji*. As a result, consumption poses a real danger to the health of consumers. The upstream empirical manufacturing process and the difficult sales conditions would increase the risk of contamination and growth of microorganisms. Thus, the competent authorities must inform and raise awareness of the health risks to consumers. A regulation of street food could limit the risk of contamination. Awareness of hygienic measures such as routine hand washing before and during production would reduce the risk of contamination. Adequate measures must be taken during manufacture to produce a less risky *Gnamakoudji*. These measures may include decontamination of the raw material with all equipment used, hot packaging, wearing gloves, heat treatment at the end of the preparation of the drink; which would limit transmissions of germs. Good communication about the dangers of eating street foods would help to improve the well-being of consumers.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**


