Original Research Article

Reduction of microbial contamination of *Chechin nchabe* (cooked fermented cassava) produced in Noun (west Cameroon) by a second fermentation

ABSTRACT

The *Chechim nchabe* is a traditional food widely consumed in Foumban, Foumbot, Koutaba, Massangam, Kouoptamo, Malentouen and Magba, 07 Departments ofNoun (West region of Cameroon). It is obtained by fermenting cassava sticks cooked on the surface of river orspring water. Unfortunately, the bad hygienic quality of the environment during production promotes its contamination by pathogenic germs. The objective of this study is to carry out a second fermentation in order to reduce contamination of *Chechim nchabe* by pathogens germs during production. To achieve this objective, a survey on the socio-economic data, profile of the producers, production protocol and characteristics of product have been realized. After microbiological analysis of *Chechim nchabe*, a second fermentation was performed in the laboratory. From the results, it appears that all the producers are women, aged between 51 and 58 years and 87% of them not attending school. The water used for soaking the cassava revealed that 54% of women use river water and 46% spring water. The *Chechim nchabe* samples collected are by enterobacteriaceae, moulds, staphylococci, *Escherichia coli* and lactic acid bacteria with respectively concentration of 4.7; 4.1; 4.4; 4.7 and 4.8 Log_{10}ufc/mL.However, *Chechim nchabe* produced in urban areas such as Foumbot and Foumban recorded low contamination compared to that produced in rural areas like Massangam, which were heavily contaminated with *Escherichia coli* and enterobacteriaceae. It was also noted that the *Chechim nchabe* produced in spring water is more contaminated than that produced in river water. The second fermentation for 10 hours of *Chechim nchabe* in a basin, after 12 hours of traditional fermentation, eliminated all of pathogenic germs from *Chechim nchabe*. This second fermentation of 10 hours could be a solution to guarantee the sanitary quality of *Chechim nchabe* before its consumption.

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Keywords: Microbial contamination, fermentation, cassava, river water, spring water

1. INTRODUCTION

The cassava is a tropical root crop, native to the Amazon basin, which provides a staple diet for about 800 million people worldwide. It is grown on 19.6 million hectares worldwide with an estimated annual production of 252.2 million tonnes and an average productivity of 12.8 million tonnes [1]. In Cameroon, the cassava sector is particular importance in the food base. It is estimated to account for 20% of cultivable land and 46% of national food production. It is one of the few staple crops that can be grown efficiently on a small scale, without the need for mechanisation or the purchase of inputs and in marginal areas with poor soils and uncertain rainfall [1]. The forms in which it is consumed vary according to town, ethnic group or region [2]. Some people prefer it as fresh root, others as processed products (*Fufu,Gari* or Sticks) [3].Despite the existence of a large number of known cassava by-products, there are still several traditional foods produced from cassava that are little known outside the region in which they are produced. This is the case of *Chechim nchabe*, a product obtained from the fermentation of cassava tuber in surface waters in the Noun Department (Cameroon). It is a food that is highly appreciated and widely consumed by the populations. Its specific characteristics are based on its organoleptic characteristics, particular purplish black color, high fibre content and easy to digest. Like cassava product, *Chechim nchabe* also promotes intestinal transit and helps relieve stomach problems, colon irritation and constipation [4]. The *Chechim nchabe* is sold in all the markets of the Department of Noun, and is often eaten as a main course and may be accompanied by black fruits (*Canarium odontophyllum*), avocado or any other complement for convenience. This product is therefore considered a key element in the fight against the problems of food insecurity that plague

households, but also as an employment-generating sector for local populations, given the easy, cheap and permanent access to raw materials for producers.

Despite the importance of *Chechim nchabe* in the dietary habits and well-being of the people of Noun, it was found that its production, like most cassava-based products, is done in a traditional condition and faces problems of good hygiene practices and good manufacturing practices. One of the most important unit operations in the production of *Chechim nchabe* is the soaking of cassava sticks obtained after cutting in untreated surface water. However, many studies have shown in the surface water the presence of pathogens such as *Salmonella*, *Campylobacter* that can be responsible for serious diseases in consumers [5]. In view of the risks incurred by consumers of *Chechin nchabe*, it is urgent to find a solution to limit the risk of contamination of this appreciated food during its traditional production in Noun (Cameroon).

2. MATERIAL AND METHODS

2.1 *Chechim Nchabe* Production Survey

First of all, survey was carried out among *Chechim nchabe* producers. The Information were collected on socio-economic profile of the producers (gender, age, schooling level, selling price of *Chechim nchabe*), the production protocol (raw material used, quantity of water use, treatment applied, fermentation time, types of surface water use) and characteristics of product (Color and taste). One of theobjectives of this survey was to identify the different factors that could contribute to the risk of contamination of the *Chechim nchabe* during production process.

2.2 Sample Collection Sites

A total of 07 Department of Noun (Cameroon) out of the 09 were selected as sample sites based on their involvement in *Chechim nchabe* production. The sites of Foumban (Latitude 5.728226 and Longitude 10.89716), Foumbot (Latitude 5.510861 and Longitude 10.638147), Koutaba (Latitude 5.651146 and longitude 10.760917), Massangam (Latitude 5.425851 and Longitude 11.001855), Kouoptamo (Latitude 5.655727 and Longitude 10.612239), Malentouen (Latitude 5.712719 and Longitude 11.117803) and Magba (latitude 5.965742 and Longitude 11.225171) were therefore chosen. The Department of Noun is located in the Western region of Cameroon and Foumban as its capital. It occupies an area of 7,687 km2 and covers 52% of the Western Region of Cameroon. Figure 1 shows the geographic location of the study sites on the map of the Noun Department.

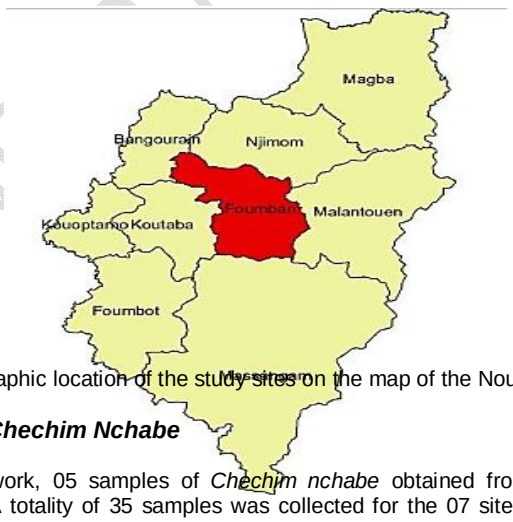

Fig. 1. Geographic location of the study sites on the map of the Noun Department [6].

2.3 Sampling of *Chechim Nchabe*

To carry out this work, 05 samples of *Chechim nchabe* obtained from different producers were collected per site. A totality of 35 samples was collected for the 07 sites studied. For each site, 03 samples from the spring water and 02 samples from the rivers water were collected in the morning. These samples have been placed in a sterile plastic bag with a zipper and transported in a cooler to the microbiology laboratory for immediate analysis. Figure 2 shows the marketed *Chechim nchabe* samples in a bucket.

Fig. 2. The *Chechim nchabe*sample

2.4 Isolation of Microorganisms in *Chechim Nchabe*

The different germs or germ groups isolated were total aerobic mesophilic flora (TAMF), enterobacteriaceae, *Escherichia coli*, staphylococci, lactic acid bacteria and moulds. For this purpose, 10 g of each sample was aseptically introduced into an Erlenmeyer containing 90 ml of sterile physiological water (NaCl 0.9 %) constituting the stock solution. Then, a series of decimal dilutions was made and 0.1ml of each dilution was plated onto Petri dishes containing the nutrient agar for TAMF enumeration. The same work was done on MacConkey agar for the enumeration of Enterobacteriaceae, on De Man, Rogosa and Sharpe agar (MRS) for the enumeration of lactic acid bacteria, on Chapman agar for the enumeration of *Staphylococci*, on Eosin Methylene Blue agar (EMB) for the enumeration of *Escherichia coli* and on Potato Dextrose Agar (PDA) supplemented with chloramphenicol for the enumeration of moulds[7]. The following formula was used to evaluate the microbial concentrations in the stock solutions.

$$\mathbf{C} = \frac{\sum \mathbf{N} * \mathbf{Fd}}{\mathbf{1,1} * \mathbf{V}}$$

With :
C : Microbial concentration (CFU/g);
N : Sum of colonies counted on Petri dishes of two successive dilution;
Fd : Dilution factor of the smallest dilution;
V: Volume (ml) seeded in the Petri dish.

2.5 Influence of the Second Fermentation on Microbial Contamination and pH of *Chechim Nchabe*

In order to guarantee the safety of *Chechim nchabe*, a second fermentation will be carried out before the marketing of *Chechim nchabe.* The soaking water used for the second fermentation and the samples of *Chechim nchabe*were collected in the soaking waters and sites that recorded the most contamination during this study. The *Chechim nchabe* sample and soaking water were collected respectively in the sterile plastic zip bags (27cm x 17cm) and sterile bottle (1L). All samples were transported to the laboratory in the cooler for start the second fermentation. Once in the laboratory, the collected *Chechim nchabe* sample was soaked in the water (1/3, m/v) contained in the sterile glass container. The preparation obtained was preserved at room temperature (25+2°C) for fermentation. During this fermentation, *Chechim nchabe* samples and soaking water were taken every 2 hours to evaluate respectively the variation of the microbial load and the pH. The figure 3 shows the *Chechim nchabe* fermentation in the glass container in the laboratory.

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Fig. 3. *Chechim nchabe* soaking operation in the laboratory

2.5.1 Microbial enumeration during second soaking

In this part of the work, the microorganisms such as lactic acid bacteria, enterobacteria, moulds, staphylococci and *Escherichia coli*that are isolated from the commercialized *Chechim nchabe*will also be searched every 2 hours during the second fermentation. The approach used for the enumeration was the same as the one described earlier in this work.

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2.5.2 pH soaking water change during second fermentation

The pH was determined according to the AOAC method [8]. At the beginning of the operation and every 2 hours, 10g *Chechim nchabe* sample was crushed, introduced into an Erlenmeyer flask and homogenised with 20 mL of distilled water. After stirring the suspension to be analysed with a magnetic stirrer, the pH meter electrode was introduced into the preparation and the corresponding pH value was displayed on the pH meter screen.

2.6 Statistical Analysis

The data obtained were analysed and represented in the form of mean using Excel spreadsheet version 2013. Sigma plot 11.0 software was used to draw the curves, histograms and XLSTAT 2007 for the Principal Component Analysis (PCA).

3. RESULTS AND DISCUSSION

3.1 *Chechim Nchabe* Production Survey

3.1.1 Socio-economic results on *Chechim nchabe* sector

The Socio-economic results shows that the main actors in this activity are exclusively women (100%), aged between 51 and 58 years. Among these producers, 87% of them not attending school and 13% have a primary level of education. The survey on the different water used for soaking the cassava revealed that 54% of women use river water while the remaining 46% use spring water. The production of *Chechim Nchabe* is between 20 and 30 kg per woman and once a week, preferably before market days. The minimum purchase price is 50F FCFA in a small bowl (200 mL). A production cost estimated between 4500 and 5000F CFA allows a profit of 85.5%.

3.1.2 Description of production of *Chechim nchabe*

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Chechim nchabe is obtained from cassava tubers previously cooked and fermented in surface of river or spring water. To produce *Chechim nchabe*, the unwashed cassava tubers are cut into several portions (10-13 cm) and introduced into a pot containing water (1/2, m/v) for cooking on a wood fire during 35 minutes at 98°C. After cooking, the tubers are peeled and cut into stick (10-13 cm long, 1-1.5 cm thick) in a container. The product obtain is then placed in a traditional woven rattan basket, to which mud collected at the bottom of the river or spring water is added. The cassava mixed with mud

was soaked for 12 hours (6 pm to 6 am) in order to give the food its main organoleptic and physicochemical characteristics. After this step, the cassava in the basket is removed and rinsed on site with river or spring water. The resulting product is then transferred to a bucket and the same soaking water in some cases is added (1/3, m/v) to the product for commercialization. At the end of this production process, the *Chechim nchabe* obtained in the form of a stick is characterized by its slightly acid, fruity taste and its purplish black color. The purplish black color of the *Chechim nchabe*, which is a quality requirement for the consumers according to the interviewed producers, is a characteristic that is obtained only when the production is carried out in river or spring waters. This requirement forces the producers to keep this traditional method of production despite the health risks for consumers. The figure 4 and 5 shows respectively the diagram and images of the traditional production of *Chechim nchabe*

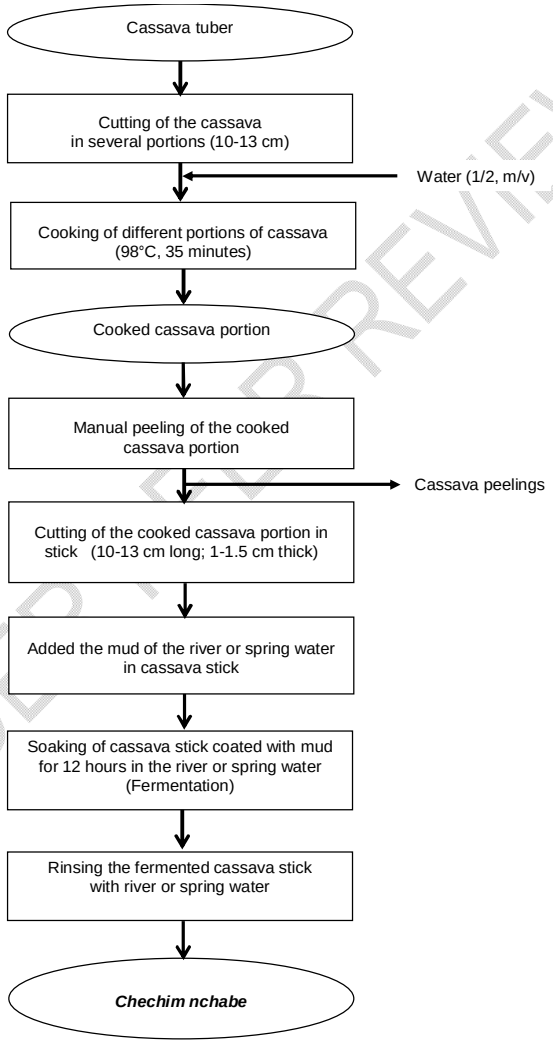
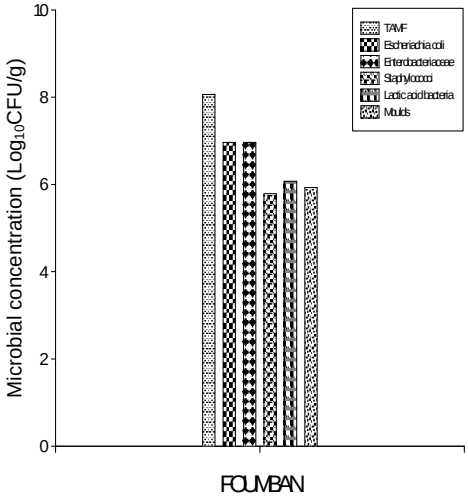


Fig. 4. Diagram of the traditional production of *Chechim nchabe*

Fig. 5. Traditional production of *Chechim nchabe* in images: Cutting of cassava in several portions (01), Cooking of cassava portions (02), Manual peeling of cooked cassava (03 and 04), Cutting cooked cassava in stick (05), Introduction of cassava in the woven rattan basket (06), Mixing cassava with mud (07), Soaking cassava in the river or spring water for 12 hours (08 and 09), Rinsing the fermented cassava (10 and 11) and *Chechim nchabe* (12)

3.2 Microbiological Analysis of *Chechim Nchabe* Samples

The figure 6 shows the different microorganisms isolated and the degree of their contamination in the *Chechim nchabe* samples collected from Foumbot, Koutaba, Massangam, Kouoptamo, Malentouen and Magba. This figure shows that, the pathogens such as *Escherichia coli,* Staphylococci, Enterobacteriaceae and moulds were all found in *Chechim nchabe* from the different sites studied. We also note the presence of Lactic acid bacteria, which are the main microorganisms involved in the lactic fermentation of *Chechim nchabe*. However, Massangan recorded the highest levels of *Escherichia coli* and moulds in *Chechim nchabe* with concentrations of 7.0 and 6.0 Log_{10}CFU/g, respectively. Magba recorded the highest concentration of Enterobacteriaceae (7.0 Log_{10}UFC/g) and TAMF (8.1 Log_{10}UFC/g) in *Chechim nchabe*. The staphylococci was higher (6.9 Log_{10}UFC/g) in the *Chechim nchabe* collected in Malentouen. As for lactic acid bacteria, the Koutaba and Kouoptamo recorded the highest concentration, with values of 7.8 Log_{10}UFC/g each. Although also contaminated, Foumban and Foumbot recorded the lowest concentrations of all studies microorganisms. Compared to the standards of the Government of Quebec on pre-cooked and fermented products, we find that these products are not suitable for consumption. In view of the results obtained, it is therefore urgent to find a solution that can reduce this bacterial load to acceptable concentrations in order to guarantee the health of consumers.


Microbial concentration (Log10CFU/g)
10
8
6
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2
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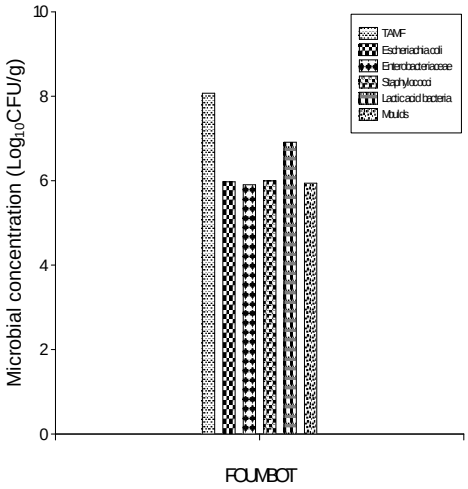
TAMF
Escherichia coli
Enterobacteriaceae
Staphylococci
Lactic acid bacteria
Moulds
FOUMBAN


Microbial concentration (Log10CFU/g)
10
8
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2
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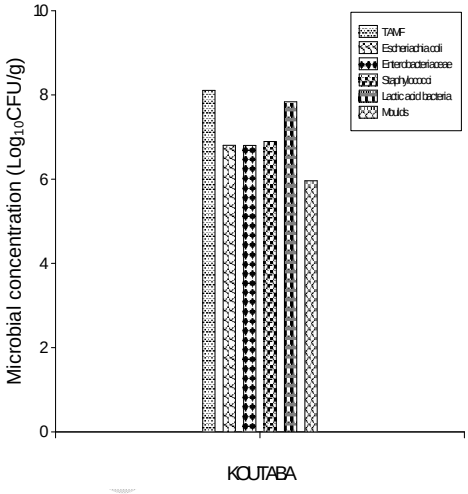
TAMF
Escherichia coli
Enterobacteriaceae
Staphylococci
Lactic acid bacteria
Moulds
FOUMBOT


Microbial concentration (Log10CFU/g)
10
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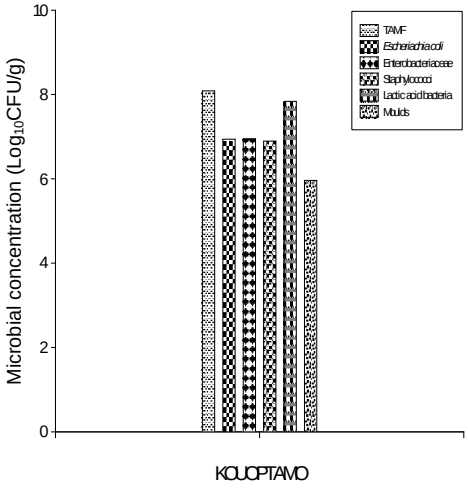
TAMF
Escherichia coli
Enterobacteriaceae
Staphylococci
Lactic acid bacteria
Moulds
KOUTABA


Microbial concentration (Log10CFU/g)
10
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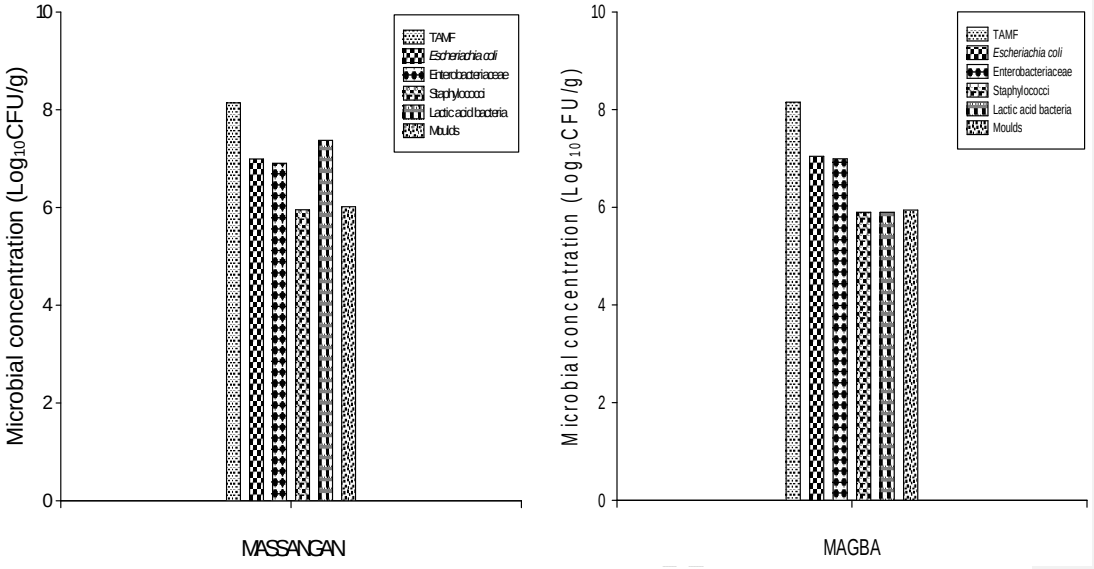
TAMF
Escherichia coli
Enterobacteriaceae
Staphylococci
Lactic acid bacteria
Moulds
KOUOPTAMO



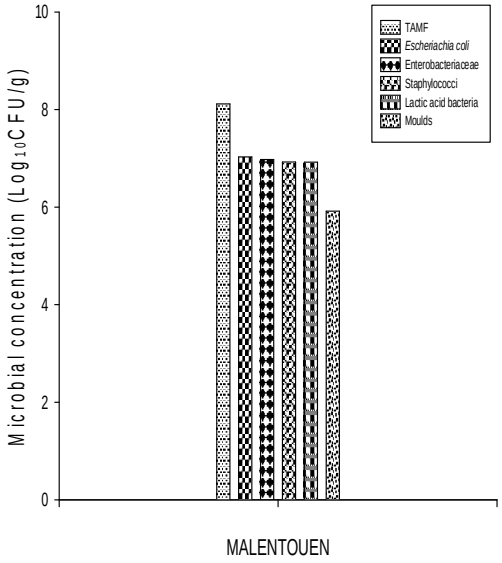


Fig. 6. Contamination of *Chechim nchabe* collected from Foumbot, Koutaba, Massangam, Kouoptamo, Malentouen and Magba by microorganisms.

3.3. Distribution of the contamination according to the sites studied

After performing a Principal Component Analysis, it can be seen that the variables TAMF*Escherichia coli*, Enterobacteriaceae, Staphylococci, lactic acid bacteria and moulds correlate to the axis system (F1xF2) at 71%. From this figure, it can be seen that TAMF, *Escherichia coli*, and Enterobacteriaceae actively participate in the formation of the axis F1 (41%) with respective contribution percentages of 20, 36, and 35%. While variables such as staphylococci, lactic acid bacteria, and mould contributed to the formation of the axis F2 (30%). Similarly, this figure shows that observations such as Foumbot (75%), Massangam (11%) contributed to the formation of the axis F1 and Kouoptamo (19%), Magba (32%) Foumban (26%) and Koutaba (20%) contributed to the formation of the axis F2 (30%). The projection of variables and observations on the F1 and F2 axes shows that *Chechim nchabe* produced in urban areas such as Foumbot and Foumban recorded low contamination with all pathogens. In contrast, *Chechim nchabe* collected in rural areas such as Koutaba and Kouoptamo recorded high contamination with staphylococci (6.8 Log_{10}CFU/g), lactic acid bacteria (8.1 Log_{10}CFU/g) and moulds (5.9 Log_{10}spores/g). The same observation was made for *Chechim nchabe* collected in Massangam, which was heavily contaminated specifically with TAMF (8 Log_{10}CFU/g), *Escherichia coli* (7 Log_{10}CFU/g) and enterobacteriaceae (7 Log_{10}CFU/g).

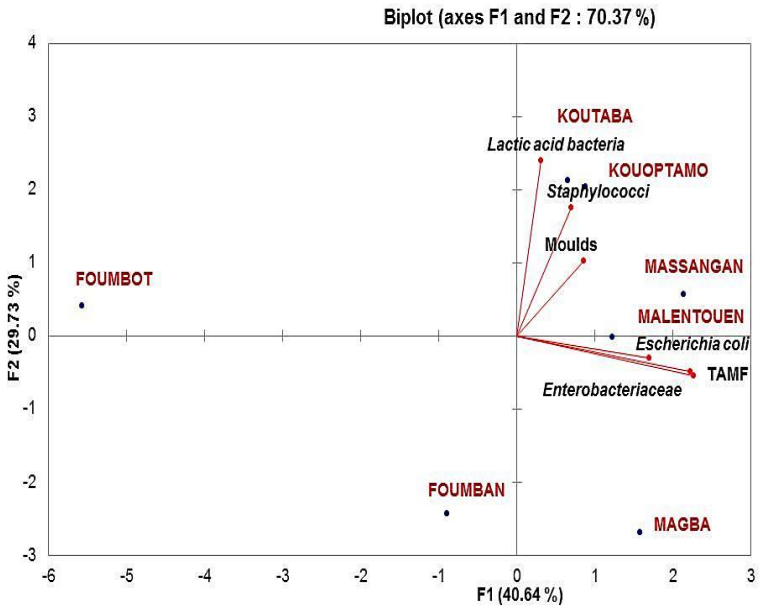


Fig. 7: Projection of observations and variables on the axis system F1xF2

3.4. Influence of the Soaking Water on Contamination of *Chechim Nchabe*

The figure 8 shows the contamination level of the *Chechim nchabe* samples according to the type of soaking water. From this figure, it can be seen that the degree of contamination in *Chechim nchabe*, regardless of the sampling site, also depends on the type of water used for soaking. However, despite the fact that this contamination is always very high in *Chechim nchabe* for all the microorganisms studied, it remains more important in the samples of *Chechim nchabe* soaked in spring water. Analyses showed that *Chechim nchabe* samples collected from spring water were more contaminated with FMAT, Enterobacteriaceae, *Escherichia coli*, lactic acid bacteria, mould and staphylococci than those collected from river water, with higher concentrations of 3.4, 6.1, 6.4, 2.2, 6.7 and 1.9% respectively. For the continuation of the work, *Chechim nchabe* samples and spring water were collected in Massangam to evaluate the influence of second fermentation on the growth of these pathogens in the laboratory.

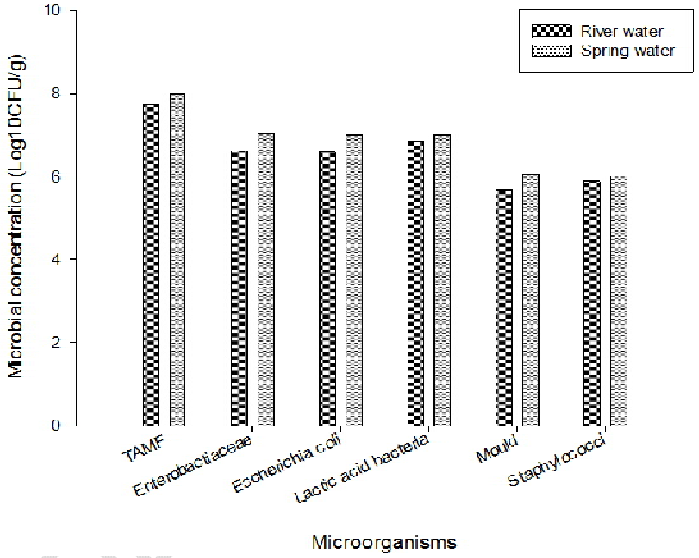


Fig. 8. Influence of the soaking water on contamination of *Chechim nchabe*

3.5 Microbial Contamination and pH Water*During* Second Fermentation of *Chechim Nchabe*

The figure 9 shows the evolution of microbial contamination and pH during the second fermentation of *Chechim Nchabe*. This figure shows that TAMF, *Escherichia coli*, staphylococci and moulds were reduced by more than 50% and enterobacteriaceae by only 31% after 08 hours of second fermentation. On the other hand, after 10 hours of second fermentation, all pathogens were completely reduced. Contrary to the pathogenic germs, the lactic acid bacteria saw their concentration doubled during this second fermentation that is an increase of 105%. During this fermentation, a progressive decrease of the pH from 6.15 to 3.71 was also observed.

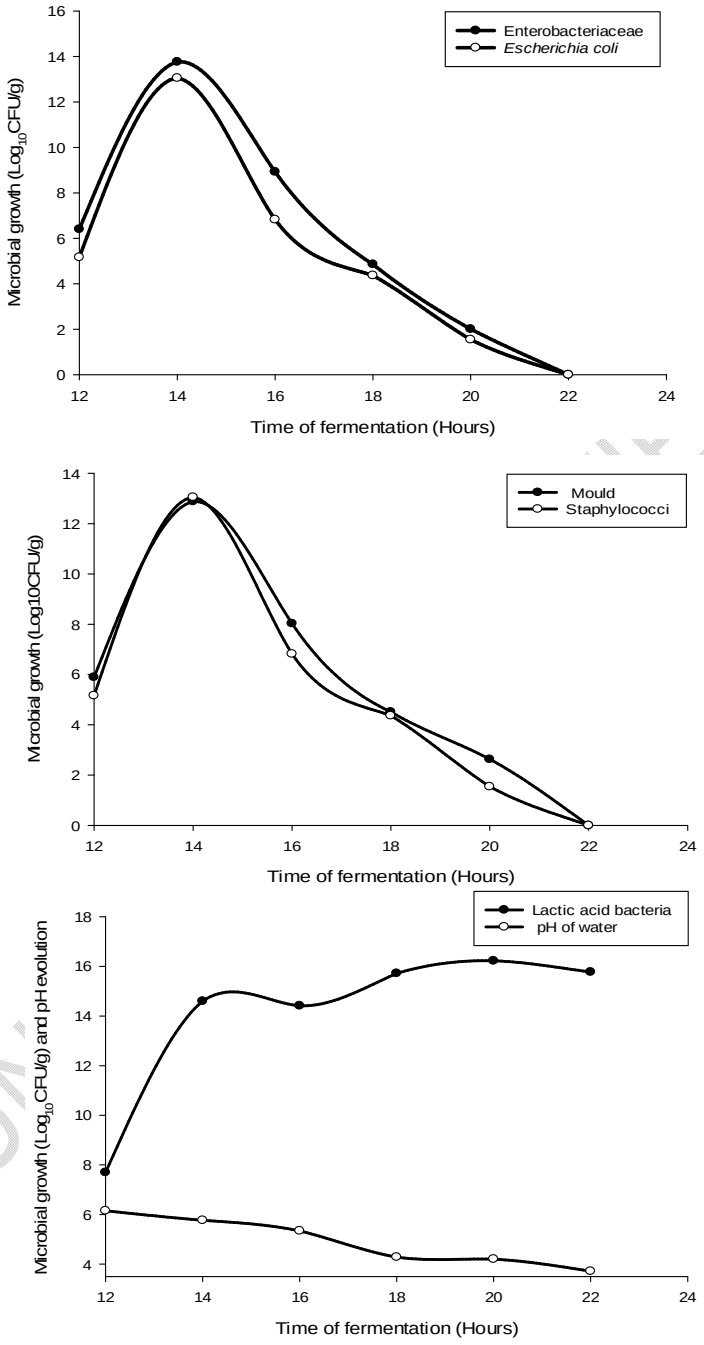


Fig. 9. Evolution of microbial contamination and pH water duringsecond fermentation of *Chechim nchabe*

4. DISCUSSION

The microbiological analysis of the *Chechim nchabe* collected in Foumban, Foumbot, Koutaba, Massangam, Kouoptamo, Malentouen and Magba revealed the presence of pathogens germs such as Enterobacteriaceae, *Escherichia coli*, staphylococci and moulds. This type of microorganisms at high concentrations is common on fermented cassava products and could be the cause of foodborne illnesses such as infections, toxin infections and food poisoning in consumers[9]. Work done by Degnon et al. [10] on the conditions of production of fermented cassava cossettes in Bassila (North-Benin) showed that all the samples analyzed had high concentrations compared to the normative criteria of TAMF, yeasts, moulds, coliforms and *Staphylococcus aureus*. This high contamination of *Chechim nchabe* noted during this work could be explained by the bad microbiological quality of the river water and spring water used both during the fermentation of cooked cassava for 12 hours and also during the marketing of *Chechim nchabe*. It was found that the *Chechim nchabe sold* at the market is soaked in basins with the same water that comes from the river or spring water used for fermentation. Indeed, the same pathogens that were originally in these fermentation sites are in permanent contact with the product ready for consumption. In fact, this microbial contamination observed in river waters and spring water is justified by the fact that they are sources of open water supply regularly exposed to all kinds of anthropological activities that promote the increase and diversification of microbial contamination[11]. Studies conducted by Djuikom et al. [12],Mbawala et al. [13] and Alia [14] respectively in Douala, Ngaoundere and in Noun revealed fecal contamination by the individual or associated presence of Coliforms, Fecal Enterococci in rivers and springs. On the other hand, some studies explain the contamination of cassava products, such as *Chechim nchabe*, by the bad personal hygiene of producers [15]. In this regard, it was observed during the surveys that the production of *Chechim nchabe* is carried out in a traditional way by undereducated (87%) or uneducated (13%) producers with little or no knowledge of the rules of Good Hygiene Practices and Good Manufacturing Practices.

Although contamination of *Chechim nchabe* was observed in all the areas studied, *Chechim nchabe* collected in rural areas such as Kouoptamo, Magba, Koutaba, Malentouen, Massangam had higher levels of contamination than those in urban areas such as Foumban, Foumbot. *Chechim nchabe* produced in urban areas is less contaminated because of direct access to potable water (tap water, borehole water, improved well water) in the homes of producers, thus limiting activities around river water and spring water. In addition, the water used for soaking *Chechim nchabe* before commercialization is often potable water.

It was also found during this work that all *Chechim nchabe* samples collected from women using spring water for soaking, whether in urban or rural areas, have higher contaminant loads than *Chechim nchabe* samples produced from river water. This result could be explained by the fact that the microbial flora present in spring waters (with low flow), thanks to its stability in these waters, clings easily to the surface of the *Chechim nchabe* during soaking. This is different from river waters (with high flow) where the microbial flora is leached partially or totally on the surface of *Chechim nchabe* by the water current, limiting the fixation of the contaminations on *Chechim nchabe.*

One of the non-pathogenic contaminants found at higher concentrations than the other flora would be lactic acid bacteria. Indeed, the presence of lactic acid bacteria is normal because they are the main microorganisms associated with cassava fermentation [16-17]due to their ability to hydrolyze cassava starch, the main food source.

After having carried out second fermentation following the 12 hours of traditional fermentation, we noted a complete inhibition of the pathogenic germs after 10 hours. Indeed,

the fermentation carried out in a closed environment (glass bottle) allowed an accumulation, a direct and prolonged action of the antimicrobial compounds such as the organic acids produced by the lactic bacteria. This is justified by the change in pH of the soaking water from 6.2 to 3.4 after 10 hours of second fermentation. Indeed, lactic bacteria have the ability to produce in their environment antimicrobial compounds such as organic acids (lactic, acetic and phenyllactic), carbon dioxide, hydrogen peroxide, diacetyl ethanol and bacteriocins that inhibit the growth of these pathogens during fermentation[18].

4. CONCLUSION

From this work, it can be seen that *Chechim nchabe* samples are contaminated with pathogens during soaking in surface waters. Although this contamination is present in all study areas and in all surface waters, it remains higher in rural areas and in spring waters. However, a second fermentation of 10 hours eliminates all pathogenic germs. This second fermentation of *Chechim nchabe* could be a solution to improving the sanitary quality of this product during production.

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