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Process and Microbiological Quality of *Soumbara* (A Local Seasoning) Used in Côte d'Ivoire

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Abstract

The present study aims at generating data that can produce useful information on the new trends in *soumbara* production processes and their effect on its microbiology quality. A descriptive survey was conducted in the production areas of Ferké, Katiola and Korhogo to determine the different production patterns. Samples of *soumbara* were collected in these production areas for microbiological analysis. The results show that regardless of the production area, there are two methods of producing *soumbara*. The differences were in the fermentation and cooking steps and the ingredients used. Microbiological analyses revealed the presence of mesophilic aerobic germs, total coliforms, *Bacillus cereus* and *Staphylococcus aureus*. The loads of mesophilic aerobic germs, *Staphylococcus aureus* and *Bacillus cereus* ranged from $(1.3\pm0.4)10^3$ to $(1.2\pm0.3)10^4$; 0 to $(1.7\pm0.8)10^2$ and $(1.1\pm0.1)10^1$ to $(4.7\pm0.2)10^1$ CFU/g, respectively. The assessment of the marketable quality of the *soumbara* samples in accordance with Directive 2005/2073/EC on microbiological criteria for foodstuffs indicated acceptable microbiological quality for the Ferké and Katiola *soumbara* samples and unsatisfactory for the Korhogo *soumbara* samples.

Keywords

Soumbara, microbiological quality, fermentation

1. Introduction

Rapid urbanization in West Africa has contributed to the development of artisanal sectors for processing and marketing local agricultural products [1]. Several traditional processing processes have been developed both by the ingenuity of farmers and through research and development in the hope of improving the organoleptic and microbiological quality of these products. Among these processes, fermentation uses the metabolism of a real microbial "machinery" for bioconversion of plant substrates [2]. In the case of the fermentation of Nere (*Parkiabiglobosa*) seeds, this process makes it possible to obtain a condiment for sauces known in West Africa as *soumbara* in Côte d'Ivoire (also called *soumbala* in Burkina Faso and Mali, *dawa-dawa* in Niger and Nigeria, *nététu* in Benin and Senegal) [3]. *Soumbara* produced in Côte d'Ivoire is a flavouring agent used to enhance the taste of sauces and dishes, and is an important source of protein, lipids, carbohydrates, vitamins and trace elements [4]. In addition, *soumbara* in general is believed to have therapeutic benefits such as regulating blood pressure, jaundice, and preventing intestinal obstruction [5]. According to [4], *soumbara*, a product resulting from the fermentation of nere seeds, contains a microbial flora of biotechnological interest. However, the manufacture of these aromatic products is a relevant process that varies according to countries and ethnic groups. Its elaboration is long (4 to 5 days) and includes three essential steps: a double cooking of the seeds, a fermentation of the

cotyledons and then a drying of the fermented product [6]. In addition, production is mainly carried out by small-scale production units, making quality control difficult. Also, preparation methods, such as the duration of fermentation, vary from one region to another or within ethnic groups, which affects the quality of the product [7].

The present study aims at generating data that can produce useful information on the new trends in *soumbara* production processes and their effect on its microbiology quality.

2. Materials and methods

2.1 Data collection and sampling

From August to November 2019, artisanal *soumbara* processors, randomly selected in 12 localities of 3 towns (Korhogo, Ferké, and Katiola) in northern parts of Côte d'Ivoire, were administered a questionnaire constructed and validated by a questionnaire expert group. Some of these cities (Korhogo, Ferké, and Katiola) are known to house people behind *soumbara* production. In collaboration with *soumbara* production units, a meeting was organized with selected groups of processors to explain them the objectives of the study. In addition, each participant was given verbal instructions on how to fill in the questionnaire and any other relevant information. The questionnaire was subjected to a preliminary validation on 15 production units to assess its clarity, the suitability of wording, and the average time needed for its completion. Based on this pilot study, necessary modifications were identified and resolved, whereas its results were not included in the final survey. The final questionnaire has been established and has been submitted to the staff on the different production site. All items were multiple-choice questions or statements with 2-6 possible answer choices including true/false and yes/no statements. The data used in this study were also collected by undisguised observations after obtaining the permission of *soumbara* production units owners to investigate and observe their practices during production in their premises. A total of 360 *soumbara* processors were included in the survey with 30 processors per locality.

After the survey, four (4) *soumbara* production units within each city were randomly selected and *soumbara* samples collected from processors within these units. In all, 12 processors were selected from the three *soumbara* producing towns and 5 samples per processor were collected from each selected production unit for microbiology analyses. All *soumbara* samples were collected from selected processors immediately after steaming in plastic bags as proposed by producers for retail selling. They were then transported in an icebox directly to the laboratory for analyses.

2.2 Sample analyses

Preparation of stock solutions, inoculation of agar plates, cultivation and quantification of micro-organisms were carried out according to [8]. For all determinations, 10 g of the samples was homogenized in a stomacher with 90 ml of sterile buffered peptone water (AES Laboratoire, Combourg, France). Tenfold serial dilutions of stomacher fluid were prepared and spread-plated for determination of micro-organism counts.

Enumeration of coliforms was carried out using plates of Violet Red Bile Lactose agar (VRBL, Merck 10660, Merck, Darmstadt, Germany). The cultures were incubated for 48 h at 30°C for total coliforms and 44°C for faecal coliforms. The eosin methylene blue agar (Becton Dickinson GmbH, Heidelberg, Germany) was used to particularly enumerate and isolate E. coli, which grows on the medium giving a distinctive metallic green sheen colony. Aerobic mesophiles were enumerated on plates of plate count agar (PCA OxoidLtd, Basingstoke, UK) and incubated at 30°C for 2 days. Identification of the organisms isolated was based on cultural characteristics, morphology of cells and biochemicaltests. The media and reagents were prepared as described by [9, 10].

Staphylococcus aureus. Staphylococcus aureus was isolated and enumerated according to the method described by [11]. A volume of 0.1 ml of each dilution was surface plated on Baird-Parker agar containing egg yolk tellurite emulsion (Oxoid) and incubated at 37°C for 24 and 48 h. The total number of colonies, colonies with a typical morphology of *Staphylococcus aureus* and colonies with different morphology to those of *Staphylococcus aureus* were counted. Five colonies from each sample were randomly selected, purified and tested for cell morphology, arrangement of the cells, Gram reaction, catalase activity, oxidase test, ability to produce acid anaerobically in a glucose-containing growth medium, coagulase activity, thermo-stable nuclease activity, acid production from mannitol and acetoin production. Only, the gram-positive cocci were identified using the identification schemes proposed by [12]. After the identification, the percentages of *Staphylococcus aureus* and the other strains were calculated. These percentages were later used to correct the results of the counts obtained from each Baird-Parker agar plate.

The method of [13] was used. The tryptone sulphite neomycine (TSN) agar (Bio-Rad, Marnes-La-Coquette, France) was used forthe detection of *Clostridium perfringens* after a thermal shock of the dilutions (80°C for 15 min and immediately cooled). One (1) millilitre of each appropriate treated dilution was used to inoculate the tryptone sulphite neomycine TSN agar (Bio-Rad) stored in surfusion at 45°C in assay tubes. After the agar had solidified, all inoculated media were incubated in an upright position for 24 h at 46°C. Tubes containing between 30 and 300 colonies were counted, and five colonies were picked for confirmation in motility-nitrate medium. The quantitative estimation of spores of *Ba-*

cillus cereus was performed by a standard plate-counting method. Isolations were achieved from heat-treated dilutions by plating on mannitol egg yolk polymyxin B agar [14]. Presumptive colonies of *Bacillus cereus* were randomly selected based on characteristic colony feature, purified on the same medium and identified by morphological, cultural and biochemical characteristics according to the documented procedures [15]. The research of *Salmonella* in attieke samples was achieved according to the procedure described in the global Salmonella surveillance and laboratory support project of the World Health Organization [16]. From each sample, 25 g was aseptically weighed and macerated in 225 ml of buffered peptone water (Oxoid) and incubated at 37°C for 24 h. A selective enrichment in Tetrathionate broth (Muller-Kauffmann) and Rappaport Vassiliadis soy peptone broth using 1 ml of previously incubated buffered peptone water was achieved at 37°C for 24 h, followed by a subcultivationon *Salmonella Shigella* agar incubation at 35°C for 24-48 h [17]. Colourless, transparent and with a black centre colonies were further identified using biochemical tests.

2.3 Data analysis

Software R. 3-01, ANOVA method with Duncan's test, significance level 5% was used. This software was used to calculate the means, standard deviation of microbiological parameters. It was also used to compare the means of the microbiological parameters of the samples and to determine whether the differences observed in the means of the microbiological parameters were significant at the 5% significance level. The survey data were processed using IBM SPSS software (Statistics 20).

3. Results

3.1 *Soumbara* production process in Côte d'Ivoire

Whatever the area of production, there are two methods of producing *soumbara*.

For method I, the *néré* pods are crushed to obtain the néré grains (Figure 1, Figure 2). The grains are washed and dried for eight (08) hours. The dried kernels are cooked for twenty-four (24) hours at 100°C (Figure 3). The grains after cooking are drained and then shelled with sand (Figure 4, Figure 5). The grains of sand facilitate dehulling. Once shelled, the grains are washed, floated and sieved to remove the sand and husks (Figure 6). The grains will be drained for 10 to 20 min and sorted to separate the unshelled and shelled grains. The shelled grains will be cooked for two to four hours. The cooked grains are placed in a basket covered with plastic film and left to ferment for 2 to 3 days in a warm place (Figure 7). The fermented grains were then covered with salt and shaped into pellets and dried for two to three days in the sun to obtain the ball-shaped *soumbara* (Figure 9) which could be ground to obtain powdered *soumbara* if desired.

For Method II, the *néré* seeds were first sorted by hand to separate the small seeds from the large ones, while separating the impure objects. These cleaned seeds were soaked in water for at least 30 minutes. The soaking softens the kernels to facilitate looting and also reduces the time of looting. The seeds are put in a mortar for shelling until the first skin is removed, the seeds are then dried this operation takes 5 hours (Figure 8). The peeled seeds were then boiled for 12 to 13 hours for a first cooking until they were easily prickly and red. The second hulling followed by washing the boiled seeds, which took 3 to 4 hours, then the seeds were then drained in a basket for 10 to 30 minutes. The seeds obtained in the previous step are put back into a pot containing water for a second cooking. The cooking process takes 1-2 hours. This step is essential because it eliminates possible pathogenic germs after the seeds have been touched several times by the hands during the treatment. The cooked Fines are put in fermentation for 40 hours. For this step, clean empty bags must be used. The seeds are poured into the empty bags with salt to speed up the fermentation process in some growers and then the bags are closed. After fermentation, the product is dried for milling to obtain powdered *soumbara* (Figure 10). On the other hand, when you want *soumbara* in granulated or ball form, you can do a pre-drying first, followed by balling. These results are shown in Figure 11.

Figure 1. Pod of *néré*.

Figure 2. Grains of *néré*.

Figure 3. Cooking the grains.

Figure 4. Drainage after cooking.

Figure 5. Grain hulling.

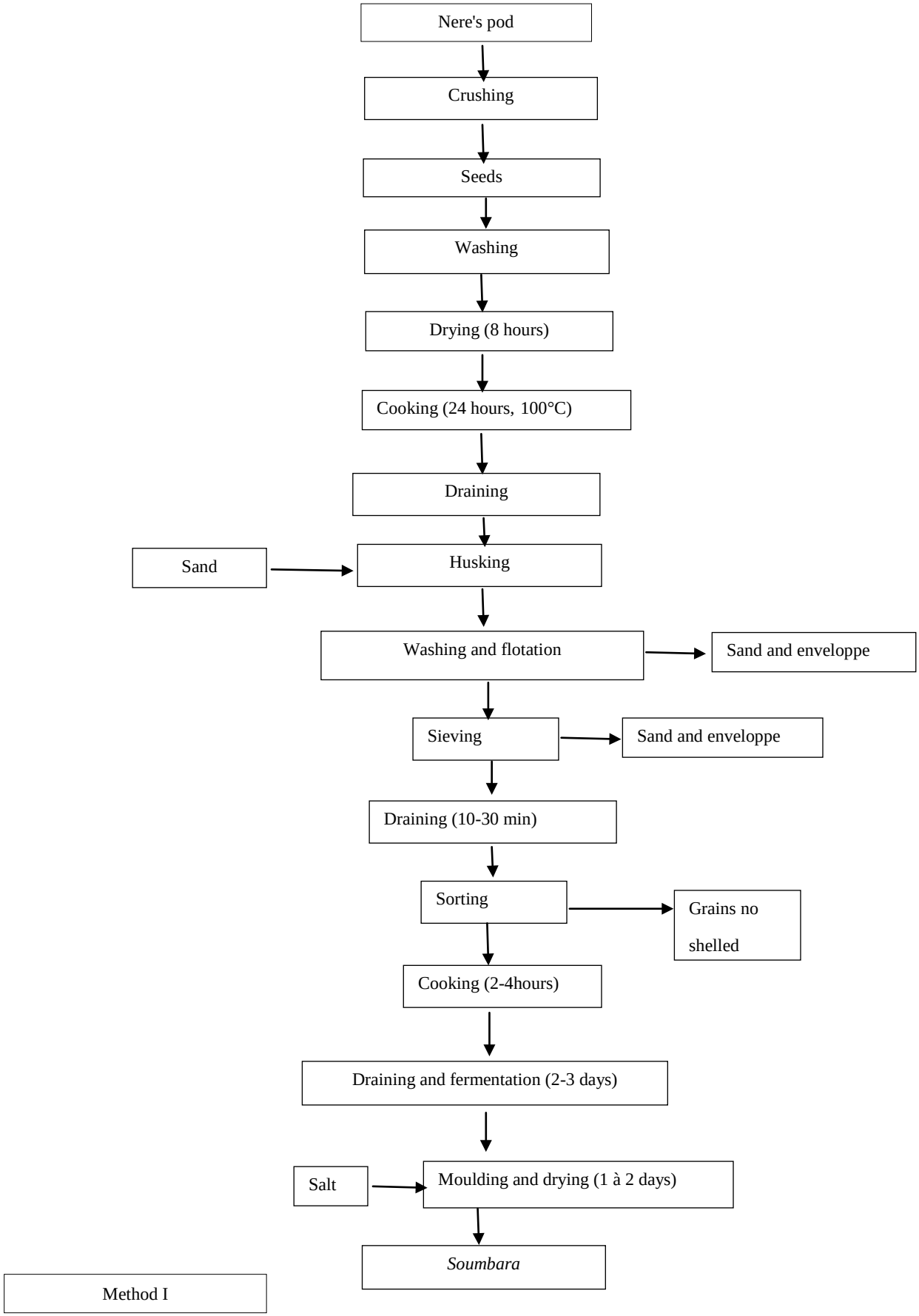
Figure 6. Washing and Drainage.

Figure 7. Fermentation.

Figure 8. Drying.

Figure 9. Moulding and drying.

Figure 10. Soumbarapowder.


Nere's pod
Crushing
Seeds
Washing
Drying (8 hours)
Cooking (24 hours, 100°C)
Draining
Sand
Husking
Washing and flotation
Sand and enveloppe
Sieving
Sand and enveloppe
Draining (10-30 min)
Sorting
Grains no shelled
Cooking (2-4hours)
Draining and fermentation (2-3 days)
Salt
Moulding and drying (1 à 2 days)
Soumbara

Method I

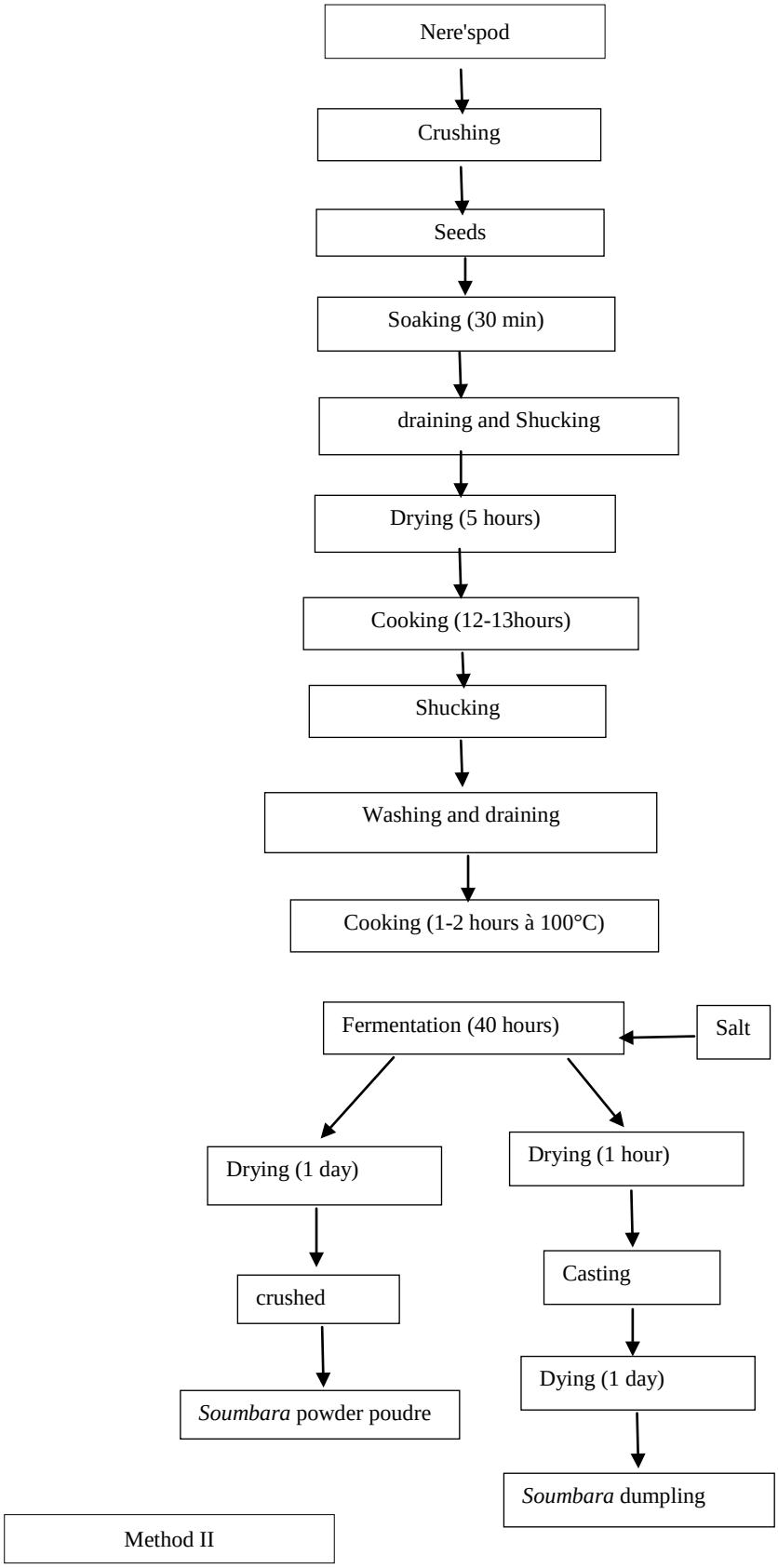


Figure 11. Production process of Soumbara in Côte d'Ivoire.

3.2 Microbial load of *soumbara* from the Korogho, Ferké and Katiola production areas

In general, microbial loads of *soumbara* varied little from one producer to another within the same production area.

Soumbara produced in the Korhogo, Ferké and Katiola production areas did not contain *Clostridium perfringens*, *Escherichia coli* and *Salmonella*. However, *soumbara* from the production areas of Korhogo and Ferké was contaminated with mesophilic aerobic germs, total coliforms, *Staphylococcus aureus* and *Bacillus cereus*. *Soumbara* from the Korogho production area was more contaminated with mesophilic aerobic germs and *Staphylococcus aureus* with loads of $(1.2\pm0.3)10^4$ CFU/g and $(1.7\pm0.8)10^2$ CFU/g respectively. The one in the Ferke production area was more contaminated by *Bacillus cereus* and coliform bacteria with respective loads of $(4.7\pm0.2)10^1$ CFU/g and $(1.9\pm0.1)10^1$ CFU/g. As for *soumbara* from the Katiola production area, it was contaminated by mesophilic aerobic germs and by total coliforms with respective loads of $(2.7\pm 0.2)10^3$ CFU/g and $(1.4\pm0.2)10^1$ CFU/g. *Soumbara* in the production areas of Ferké and Katiola are of satisfactory microbiological quality while Korhogo is of unsatisfactory microbiological quality because its mesophilic aerobic germs load was higher than the 2005/2073/EC standard (Table 1).

Table 1. Microbial load (CFU/g) of *nere soumbara*

Germs (CFU/g)	Production zones			
	Korhogo	Ferké	Katiola	Seuil
GAM	$(1.2\pm0.3)10^{4a}$	$(1.3\pm0.4)10^{3b}$	$(2.7\pm0.2)10^{3b}$	10^6
Staphylococcus aureus	$(1.7\pm0.8)10^{2a}$	$(1.8\pm0.9)10^{1b}$	0	10^2
Bacilluscereus	$(1.1\pm0.1)10^{1a}$	$(4.7\pm0.2)10^{1a}$	$(1.4\pm0.2)10^{1a}$	-
Total coliforms	$(1.8\pm0.1)10^{1a}$	$(1.9\pm0.1)10^{1a}$	0	10^3
Clostridium perfringens	0	0	0	
E. coli	0	0	0	
Salmonella	absence	absence	absence	absence

On the same line, loads bearing the same letter have no significant difference at the 5% threshold, GAM: Mesophilic Aerobic Germs; E. Coli: Escherichia coli;

4. Discussion

Soumbara production process was examined in the production areas of Korhogo, Ferké, and Katiola in northern Côte d'Ivoire with the aim of generating data that could provide useful information on the *Soumbara* production processes and how the microbiological quality of the products was affected and/or generated in the main processing areas. It was found that regardless of the production area, there were two production methods. The two methods differed in terms of cooking and fermentation times. This would be explained by the fact that the production areas are located in the north of the country and that these peoples have been producing this food since the dawn of time and the know-how is faithfully transmitted from generation to generation. Microbiological examination made it possible to assess the level of contamination of *soumbara* by non-fermentative microorganisms. The high load of mesophilic aerobic germs would favour a strong spoilage of *soumbara* and would constitute a risk of pathogenic germs [18]. This alteration flora, consisting of the total mesophilic aerobic flora with the highest content $(1.2\pm 0.3)10^4$ CFU/g would be of the same order of magnitude as $(18.47)10^4$ CFU/g reported by [19], in Guedji, a condiment based on fermented and dried fish. To this would be added *Bacillus cereus*, total coliforms and *Staphylococci aureus*. These microorganisms could degrade the products by altering the taste, smell, appearance, in short the marketable quality of *soumbara* [20]. The study indicated the absence of *Salmonella* and Anaerobic Sulfito-Reducing Anaerobes that would be due to the fermentation that makes the environment hostile to their growth [2]. The market quality assessment of the *soumbara* samples according to Directive 2005/2073/EC on microbiological criteria for foodstuffs indicated an acceptable microbiological quality for the Ferké and Katiola *soumbara* samples. On the other hand, due to their levels of *Staphylococcus* above the maximum acceptable value (100 CFU/g), the *soumbara* from Korhogo would be of unsatisfactory microbiological quality. This high load of *Staphylococcus* would be due to exposure to the open air during drying or sale but could also come from the hands of producers. However, corrective measures could help to reduce the *Staphylococcus* load. The absence of *Salmoella, Clostridium perfringens* and *Echerichia coli* in the samples would be the action of fermentative germs such as lactic acid bacteria that during fermentation not only produce bacteriocins but also acidify the environment which becomes unfavourable to these germs [21].

5. Conclusion

The present study aims at generating data that can produce useful information on the new trends in *soumbara* produc-

tion processes and their effect on its microbiology quality. This study revealed that in Côte d'Ivoire there are two methods of *soumbara* production processes for *nere* in all production areas. *Soumbara* produced was contaminated with mesophilic aerobic germs and total coliforms but did not contain *Clostridium perfringens*, *Escherichia coli* and *Salmonella*. The assessment of the marketable quality of the samples of *soumbara* in accordance with Directive 2005/2073/EC on microbiological criteria for foodstuffs indicated an acceptable microbiological quality for the samples of *soumbara* from Ferké and Katiola and unsatisfactory for the samples of *soumbara* from Korhogo.

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Competing interests

Authors have declared that no competing interests exist.

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