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Assessment of the Microbial Contamination of Millet Flour (*Pennisetum glaucum*) Sold on the Public Markets of Daloa, Côte d'Ivoire

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Abstract The millet flour sale is a very common activity in the markets of Daloa city. Despite its great consumption and its many uses by the Ivorian populations, this millet flour is produced in an artisanal or semi-artisanal way in an uncontrolled environment. This leads to various microbial contaminations thus impacting this flour quality. Our study aimed to assess the microbial contamination of millet flour sold in public markets in Daloa city. A production survey was carried out in order to obtain information on the personal hygiene of the saleswomen as well as the sale conditions. During this study, some physico-chemical and microbiological parameters of 45 flour samples (9 samples per market) were analyzed. The results revealed that the millet flour sale is exclusively carried out by young women (54.5%). In the majority of cases, these women are illiterate (72%) and have inadequate personal hygiene (55%) and an unacceptable sales environment (79%). The millet flour samples are highly contaminated and have a poor microbiological quality. In addition, certain pathogenic species such as coagulase positive *Staphylococcus aureus, Escherichia coli* and *Bacillus cereus* have been identified in the flour sold in the Daloa markets. Additionally, pH and humidity varied from sample to sample and from market to market. The presence of these germs would reflect a lack of good millet manufacturing practices, which would represent a danger for consumers. The millet flour sold in the markets would represent a danger for the population consuming millet flour in Daloa city.

Keywords: *flour, millet, microbial contamination, cereals*

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1. Introduction

Grain products occupy a central place in the agricultural system on a global scale. With a global production of around 2 billion tonnes, cereals are considered to be the main foods for human and animal nutrition [1,2]. The millet (*Pennisetum glaucum*) is one of the most important cereal crops in the arid and semi-arid regions of India and sub-Saharan Africa [3]. It is grown as a source of nutrient-rich food grain for humans as well as a forage crop for livestock [4]. In these regions, millet is the staple food of more than 90 million people, so it plays a crucial role in food security [5]. Millet is the 7th largest cereal in the world. It is the Sahelian culture par excellence because of its adaptation to the particular conditions of production in this region [6]. Global millet production now exceeds 32 million tonnes [2]. Millet is cultivated in arid and semi-arid regions of Africa primarily for human food and secondarily as fodder and building material [7,8]. Thus, millet is subject to multiple uses. These uses range from the processing of grains as a food or medicinal resource to the valorization of straw (thatch) as works of art, fodder, biofuel or as firewood [9]. In Africa, millet grains are often ground into flour, rolled into large balls, parboiled, and then consumed as porridge with milk; sometimes millets are prepared as beverages [10]. In Côte d'Ivoire, millet is the basis of several (foods) dishes including couscous, donuts, alcoholic and non-alcoholic drinks and infant food substitutes [11]. In public markets, millet already ground is very often packaged or sold in bulk on shelves. This millet flour, like many local products, is very often produced mainly using an artisanal or semi-artisanal process in uncontrolled environments [12]. Such conditions characterized by the diversity of the

transformation processes, the non-mastery of the production systems can lead to various contaminations, in particular microbial contamination, thus impacting the quality of this flour. Daloa, the third most populous city in Côte d'Ivoire after Abidjan and Bouaké, with a population of more than 245,350 inhabitants, is today experiencing profound upheavals in lifestyles, a diversity of socio-professional and economic activities which crystallize the problem of food security [13,14,15]. Like other public markets in large cities in Côte d'Ivoire, millet flour is also sold in bulk in containers without covers displayed on tables in the markets of Daloa city. Despite the high consumption of millet flour and its many uses by Ivorian populations, there is little scientific data on artisanal flours sold in public markets in Daloa.

So this work aims to assess the microbial contamination of millet flour (*Pennisetum glaucum*) sold in public markets in Daloa (Côte d'Ivoire).

2. Material and Methods

2.1. Sampling

The samples analyzed during this study consisted of millet flour collected at five different market in Daloa city. Indeed, three different samples were taken from three different vendors in the same market. A total, 45 samples were taken and analyzed during the present study at a rate of 9 samples per market. Each sample consisted of approximately 150 g of millet flour. The samples were taken under the conditions of sale of millet flour in the markets and the samples taken were collected in sterile containers and transported immediately in an icebox directly to the laboratory for analyses.

2.2. Physico-chemical Analysis

2.2.1. pH Determination

The pH of millet flour was determined using a pH meter (Microprocessor pH meter, pH 211, HANNA Instruments) according to the AOAC method [16]. The instrument was calibrated using two buffer solutions at pH 7.0 and 4.0 and this was systematically done before pH measuring. Indeed, forty grams of inoculum samples were ground in 300 ml of distilled water in a porcelain mortar and then centrifuged at 4000 tours/min for 30 min. The measurement was made by immersing the electrode in 50 ml of the supernatant and the reading is repeated three times.

2.2.2. Moisture Determination

The moisture content was determined using the method of Kimaryo [17]. The product is desiccated at a temperature of 105 ± 2°C, in a ventilated isothermal oven, at atmospheric pressure, to a constant mass. The water content is defined as the loss of mass undergone under the measurement conditions. Indeed, Porcelain crucibles are dried in an oven (digital display Binder) for 15 minutes at 105 ± 2°C. They are then left to cool in a desiccator for 30 minutes and then weighed (m_1). These crucibles containing 5 g of sample are reweighed (m_2) and placed in an oven (105 ± 2°C) for 24 hours. They are weighed (m_3) after cooling in the desiccator. The measurements are carried out in triplicate. The water content (ρ) expressed as a mass percentage of crude substance calculated from the following formula:

$$\rho = \frac{m_2 - m_3}{m_2 - m_1} x100$$

ρ = Moisture content
m_3 = mass of empty crucible
m_2 = mass of crucible + 5 g of sample
m_3 = mass of crucible + 5 g of sample placed in an oven (105 ± 2°C) for 24 h

2.3. Enumeration of Microorganisms

Preparation of stock solutions, inoculation of agar plates, and cultivation and quantification of microorganisms were carried out according to Coulin method [18]. For all determinations, 10 g of the sample were homogenized in a stomacher with 90 ml of sterile diluent containing 0.85% NaCl and 0.1% peptone (Difco, Becton Dickinson, Sparks, MD, USA). Tenfold serial dilutions of stomacher fluid, ranging from 10^1 to 10^7, were prepared and spread-plated for the determination of microbial counts. So, Plate Count Agar (PCA) was used to count aerobic mesophilic flora at 30°C for 72 hours as recommended in NF/ ISO 4833: 2003. Enterobacteria count was performed at 37°C for 24 h on Violet Red Neutral Bile Glucose (VRBG) agar according to ISO 21528-2: 2004. Violet Neutral Bile Lactose (VRBL) agar was used for fecal coliforms count at 44°C for 24 h as described in ISO 4832: 2006. Typical Enterobacteria and fecal coliforms colonies were confirmed by oxidase test. Yeasts and molds were counted with Sabouraud agar containing chloramphenicol 25°C for 5 days according to the NF/ISO 16212: 2011 standard. *Staphylococcus aureus* was identified using Baird-Parker Agar containing Telluride Egg Yolk and 0.2% Sulphamethazine at 37°C for 48 h according to the French standard NF/ISO 6888: 2004. Black colonies with a clear halo (action of lecithin) and an opaque zone (action of lipase) were counted (15-150 characteristic colonies) according to dilution. Colonies were examined microscopically, tested for Gram and catalase reactions, and confirmed by coagulase activity (rabbit plasma-EDTA, Merck). Rapid'E coli 2 agar served for *Escherichia coli* isolation and enumeration at 44°C for 24 to 48 hours as recommended in standard NF/ISO 16140: 2013. Fecal streptococci were detected at 37°C for 24 h on Esculin Azide Bile Agar according to standard NF/ISO 7899-1: 1984. Bacillus cereus was enumerated and identified using Mossel agar containing a sterile egg yolk emulsion at 30°C for 24 to 48h according to standard NF/ISO 7932: 2004. Only the rough, dry, pink (mannitol negative) colonies surrounded by a pink precipitation halo and a transparent area indicating the production of lecithinase will be taken into account for the enumeration.

2.4. Colony enumeration

Colony Forming Units per milliliter of sample (CFU/ g) were calculated according to standard NF/ISO 7218: 2007 using the following formula:

$$N = \frac{\sum C}{d\left(n_1 + 0{,}1n_2\right)V}$$

ΣC: 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/g).

2.5. Microbiological Quality Assessment Standards

The results of the microbiological analyzes obtained are compared with the reference criteria to assess the safety of the sample analyzed. The results are interpreted according to a two-class design (satisfactory when the value of the microbial load is less than or equal to the reference criterion, and unsatisfactory when this value is greater than the criterion). The standards for the assessment of the microbiological quality of the millet flour produced were taken from the compendium of microbiological criteria applicable to foodstuffs - Guidelines for the interpretation, 2015 of Luxembourg, supplemented by the normative reference of the microbiological criteria of foods of Man (CE n ° 2073/2005).

2.6. Determination of the Origin of Fecal Contamination

It is based on the criteria defined by Borrego & Romero method [19]. According to these authors, the contamination is of animal origin if the fecal coliform / fecal streptococci ratio indicates 0.7 and of human origin if this ratio is greater than 4 (Table 1).

Table 1. Criteria for determining the origin of fecal contamination

Faecal coliforms / faecal streptococci ratio	Source of contamination
R < 0,7	Strictly of animal origin
0,7 < R < 1	Mixed predominantly animal
1 < R < 2	Uncertain origin
2 < R < 4	Mixed predominantly human
R > 4	Strictly of human origin

Source: [19].

2.7. Statistical Analysis

All trials were repeated four times. The different sample treatments were compared by performing one-way analysis of variance on the replicates at a 95% level of significance using the Statistica (99th Ed, Alabama, USA) statistical program. Unless otherwise stated, significant results refer to P < 0.05. This software was also used to calculate mean values and standard deviations of the trials.

3. Results

3.1. Characteristics of the Sale of Millet Flour in the Daloa Markets

3.1.1. Profile of Millet Flour Sellers

The sale of millet flour in these markets is carried out only by women (100%). This population is dominated at 33.3% by adults aged 30 and over. Young people aged 18 to 30 represented 54.5% and those aged 10 to 18 represented 12%. The survey revealed that 72% of sellers surveyed had no education. A proportion of 12% had a primary education level, 12% had a secondary education level and 3% declared having some higher education (Table 2).

Table 2. Sociodemographic characteristics of millet flour sellers in Daloa

Characteristics	People surveyed Number	Fréquence (%)
Gender		
Female	33	100
Age		
10-18 years	4	12.1
18-30 years	18	54.5
≥30 years	11	33.3
Study level		
Unschooled	24	72.7
Primary	4	12.1
Secondary	4	12.1
Superior	1	3.1

3.1.2. Staff hygiene and Sanitary Environment of the Sale of Millet Flour in the Markets

The survey showed that 79% of all millet flour seller visited had an unacceptable sales environment. In 12% of sellers, the sales environment is healthy and unhealthy in 9% (Figure 1).

In addition, more than half (55%) of flour vendors have poor personal hygiene compared to 45% who have acceptable hygiene (Figure 2).

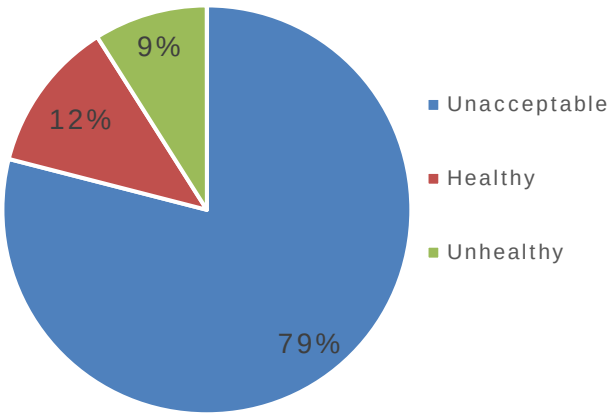

Figure 1. Sanitary environment of places where millet flour is sold

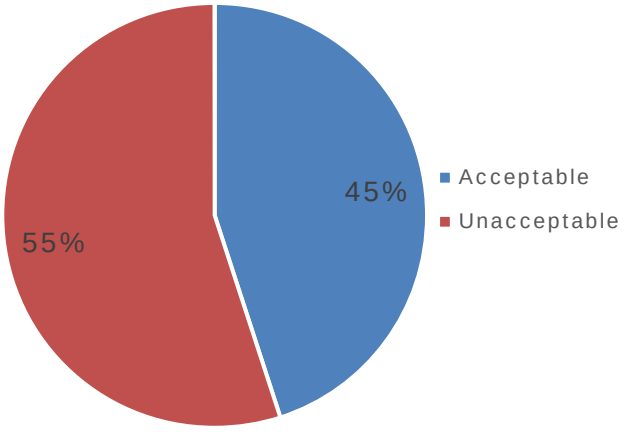

Figure 2. Personal hygiene of millet flour sellers

3.1.3. Millet Flour Use

Investigation reveals that millet flour was used for several purposes. Thus it could be used for the preparation of dishes such as *Bahka* (44%), *Tho* (28%), *Wommi* (9%) and other undefined uses (19%) (Figure 3).

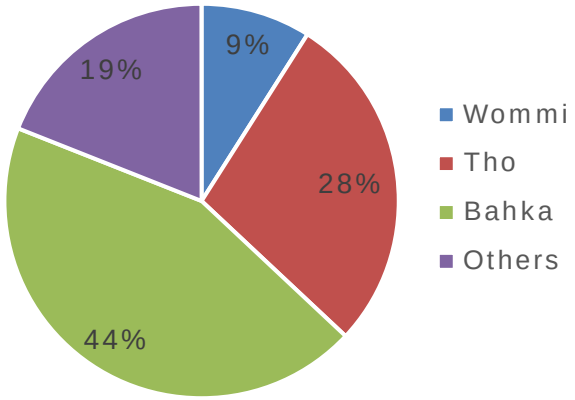

Figure 3. Use of millet flour intended for consumption

3.2. Change of pH and Moisture Content

The pH of the millet flour sold in the different markets ranged from 4.55 ± 0.06 to 6.46 ± 0.42. Millet flour from the Big market with a value of 6.46 ± 0.42 had the lowest pH while that from the Abattoir 2 market was the highest with a value of 6.73 ± 2.23 (Table 3). The moisture levels of millet flour sold in different markets varied from market to market. These rates ranged from 17.33 ± 0.47 to 39.73 ± 0.09. The highest rate was 39.73 ± 0.21 from samples from the Big market while the lowest rate was from samples from Kennedy market with a value of 17.33 ± 0.47 (Table 3).

Table 3. Moisture content and pH of millet flour samples

Markets	Moisture content	pH
Lobia 2	30.72 ± 0.40 ^{b}	5.90 ± 0.14 ^{ab}
Abattoir 2	22.20 ± 0.42 ^{c}	6.46 ± 0.42 ^{a}
Orly	32.12 ± 0.30 ^{b}	4.89 ± 0.63 ^{b}
Big market	39.73 ± 0.09 ^{a}	4.55 ± 0.06 ^{b}
Kennedy	17.33 ± 0.47 ^{d}	5.90 ± 0.05 ^{ab}

3.3. Enumeration of Microbial Flora of Millet Flour Sold in the Various Markets of Daloa

3.3.1. Flora of Alteration and Deficit of Good Hygiene Practices

Different microbial flora have been found in the millet flour sold in the different markets of Daloa. These are microflora of alteration and certain flora suggestive of a deficit in good hygiene practices. These are the fungal flora (yeasts / molds), aerobic mesophilic bacteria and enterobacteria. All the samples from the study markets were heavily contaminated with these different microflora. In addition, all the loadings of these microflora were all above the expected microbiological quality standards except the abattoir market which has an enterobacteria lower load than the standard. The loads of the aerobic mesophilic microflora varied from 6.26 ± 0.25 to 10.00 ± 0.80 log CFU/g while the standard provides only 5 log CFU/g. For fungal flora, the loads varied from 2.84 ± 0.03 to 8.25 ± 0.17 log CFU/g while the standard provides for the value of 3 log CFU/g. As for the Enterobacteria, the loads oscillated from 3.02 ± 0.38 to 3.50 ± 0.56 CFU/g whereas the standard provides for the charge 1 log CFU/g (Table 4).

Table 4. Enterobacteria, yeasts and mold and aerobic mesophiles flora (Flora of Alteration) population in millet flour samples

Markets	Enterobacteria	Yeasts and molds	A.M.
Lobia 2	3.50 ± 0.56 ^{a}	2.84 ± 0.03 ^{b}	6.26 ± 0.25 ^{c}
Abattoir 2	<1	6.42 ± 1.00 ^{a}	7.20 ± 0.34 ^{bc}
Orly	3.04 ± 0.09 ^{a}	6.90 ± 0.60 ^{a}	8.55 ± 0.06 ^{ab}
Big market	3.28 ± 0.13 ^{a}	7.17 ± 0.44 ^{a}	9.06 ± 0.71 ^{ab}
Kennedy	3.02 ± 0.38 ^{a}	8.25 ± 0.17 ^{a}	10.00 ± 0.80 ^{a}

3.3.2. Microflora of Fecal Origin

All of the millet flour samples were heavily contaminated with fecal coliforms and fecal streptococci. All the loads were above the expected microbiological quality standards, except the fecal coliform and fecal streptococcus loads from the abattoir market which were below the standards. The loads of fecal coliforms varied from 2.64 ± 0.48 to 4.50 ± 0.52 log CFU/g of millet flour while the standard provides only 2 log CFU/g. Those of fecal streptococci oscillated between 3.75 ± 0.02 and 5.15 ± 0.03 log CFU/g of millet flour (Table 5). In addition, the ratio of loads of fecal coliforms and fecal streptococci (CF/SF) in millet flour sold in the different markets of Daloa is 0.50. This report would indicate that these fecal flora are of strictly animal origin (1>CF/SF>0.7).

Table 5. Fecal streptococci and fecal coliforms (Flora of faecal origin) population in millet flour samples

Markets	Fecal streptococci	Fecal coliforms
Lobia 2	5.15 ± 0.03 ^{a}	4.50 ± 0.52 ^{a}
Abattoir 2	<1	<1
Orly	5.11 ± 0.03 ^{a}	3.48 ± 0.61 ^{a}
Big market	3.75 ± 0.02 ^{c}	2.64 ± 0.48 ^{a}
Kennedy	4.26 ± 0.18 ^{b}	3.17 ± 0.19 ^{a}

3.3.3. Potentially Pathogenic Species

Millet flour sold in various public markets contains potentially pathogenic bacterial species, in particular

Escherichia Coli, *Bacillus Cereus* and *Staphilococcus Aureus*. In addition, the presence of these three bacterial species was noted at the same time in all the samples with very high loads. These loads are also higher than those prescribed in the reference standards expected microbiological quality standards except the abattoir market which has an *Escherichia Coli* lower load than the standard. The loads of *Escherichia Coli* varied from 2.13 ± 0.01 to 6.08 ± 0.08 log CFU/g while the standard provides for only 1 log CFU/g. Those of *Staphilococcus Aureus* oscillated between 3.32 ± 0.17 and 5.67 ± 0.66 log CFU/g while the standard provides only 2 log CFU/g. The loads of *Bacillus Cereus* varied from 7.18 ± 0.06 to 10.25 ± 0.16 log CFU/g while the standard provide 5 log CFU/g (Table 6).

Table 6. *Escherichia Coli, Bacillus Cereus* and *Staphilococcus Aureus* (Potentially pathogenic species) population in millet flour samples

Markets	*Escherichia Coli*	*Bacillus Cereus*	*Staphilococcus Aureus*
Lobi 2	6.08 ± 0.08 ^{a}	7.18 ± 0.06 ^{d}	3.32 ± 0.17 ^{b}
Abattoir	<1	9.35 ± 0.30 ^{b}	4.42 ± 0.20 ^{ab}
Orly	2.46 ±0.46 ^{c}	8.41 ± 0.04 ^{c}	5.09 ± 0.88 ^{ab}
Big market	2.13 ± 0.01 ^{c}	7.38 ± 0.34 ^{d}	5.38 ± 0.19 ^{a}
Kennedy	4.07 ± 0.09 ^{b}	10.25 ± 0.16 ^{a}	5.67 ± 0.66 ^{a}

4. Discussion

Millet flour is widely consumed in Daloa town. According to the results of the survey, this flour is consumed in the form of *Bahka* (44%), *Tho* (28%), *Wommi* (9%) and other forms (19%) (Figure 3). This millet flour is sold in most of the public markets (Lobia 2, Abattoir 2, Orly, Big market and Kennedy) in Daloa city. This activity, like most food sales activities in public markets, is still the preserve of women (100%). Women, whose age varies between 18 and 30, are the main actors (54.5%) in the marketing of millet flour in public markets (Table 2). This activity would represent a main source of income for the majority of them. According to the work of Dieye [20] and those of Malete [21], informal activities such as the sale of food products by women contribute to the food security of urban populations and to the creation of jobs for these vulnerable layers. Millet flour is sold in the markets without any hygienic precautions (Figure 1 and Figure 2). The flour is displayed in bowls or jars without lids. Most of the vendors are either illiterate (72.7%) or very poorly educated (12.1%) and they have no concept of good hygiene practices for food products (Table 2). The informal nature of the sales activities, the high level of illiteracy and the lack of training programs on good food practices could justify the behavior of these ladies who are not aware of the risks of contamination of the flour on display without any precaution. These practices would make millet flour subject to several types of contamination, including that of microbial origin. These results are in agreement with those obtained of N'goran-Aw [22] during their studies on white corn flour sold in Abidjan city (Côte d'Ivoire). In this study, some physicochemical parameters (pH and humidity) were analyzed. So, the pH of the millet flour taken from the different markets varied from 4.55 ± 0.06 to 6.46 ± 0.42 (Table 3), and the majority of the samples taken during this study had a pH below 5. These results are appreciably similar to those obtained by Tchekessi [23] in millet flour during the manufacture of the pellets of cereals for the production of a fermented drink of probiotic type consumed in Benin. This phenomenon is linked to the fermentation which takes place in these millet flours. Indeed, fermentations of food products are very complex processes which normally involve interactions between plant or animal tissues and a group of microorganisms. This means that any changes that occur during fermentation would depend on the availability of nutrients and nutrient precursors in the raw material, the metabolic activities of the fermentation microorganisms and any possible interactions between these elements [24]. The majority of traditional cereal-based products consumed in Africa are transformed by natural fermentation and used particularly as food for children but also as food for adults. Cereal fermentation is also well known to have an influence on the characteristics of foods, in particular on the digestibility of proteins and starch and the bioavailability of minerals [25]. Thus, during the process of transforming millet grains into millet flour, there is a hydration step. During this stage, who can last up to 16h of time, there is a fermentation. We then witness a significant acidification reflected by this drop in pH observed at the level of certain samples taken at the level of the various markets. These results are in accordance with those of Mugula [26] who showed the decrease in pH during the fermentation of maize, sorghum and millet for the manufacture of togwa (Tanzanian fermented food). This variation in pH has also been indicated in the lactic fermentation of many food products including millet [27] and also in *Doklu*, a fermented food made from corn [28]. This phenomenon is associated with the metabolic activity of the various microorganisms of fermentation, in particular with the combined effect of bacteria and yeasts in fermented cereals as indicated by Zhang [29], but more particularly and above all with that lactic acid bacteria. Indeed, these lactic acid bacteria produce organic acids (mainly lactic acid) during their development. These acids are therefore responsible for the drop in pH during fermentation of millet during the transformation process. Similar observations have been reported by Nche [30] during their studies on the fermentation of *kenkey*, a traditional fermented food made from maize in Ghana and by Assohoun [28] in Côte d'Ivoire during the production of *Doklu*, a Fermented Maize Based Food. It should be noted that during this study, the moisture content of the millet flour taken from the different markets, namely Lobia 2, Abattoir 2, Orly, Big market and Kennedy was determined. So, moisture content ranged from 17.33 ± 0.47 to 39.63 ± 0.09. These moisture content are consistent with those obtained from flours resulting from traditional wet processing, which have between 22 and 46% moisture [31]. These results are in agreement with those of N'goran-Aw [22] who obtained a humidity level of between 21.0 and 39.7 during their studies on white corn flour sold in the markets of 9 municipalities in Abidjan city. The high moisture levels in artisanal flours had already been reported in other studies such as those of Berghofer [32] on wheat flour in Australia. These high water contents could be explained by the artisanal

production process of these flours, which means that the extraction rate is increased by a certain level of moisture in the cereal seeds. Indeed, most traditional flour mills, use second-hand equipment whose performance is severely reduced, which has the consequence of passing the product to be ground through the mill several times if the latter is not properly hydrated. Moreover, the process of transforming corn seeds into flour involves a hydration step of the latter of about 16 hours according to N'goran-Aw [12]. Studies have shown that a soaking time, between 12 and 18 hours, positively influences the degree of friability of corn kernels [33]. In addition, most flours produced in an artisanal way are intended to be sold and consumed on the day of production. When they need to be stored, users do additional drying in the sun or shade to extend shelf life. This type of drying therefore has a low impact on the humidity level. Otherwise, the microbiological analyzes carried out during this study revealed that all of samples analyzed were highly contaminated by contaminants microorganisms with maximum loads of 10.00 ± 0.80 log CFU/g and 8.25 ± 0.17 log CFU/g at the Kennedy market respectively for aerobic mesophilic microflora and yeasts and molds (Table 4). The maximum loads in enterobacteria (3.50 ± 0.56 log CFU/g), in fecal coliforms (4.50 ± 0.52 log CFU/g) and in fecal streptococci (5.15 ± 0.03 log CFU/g) were found at Lobia 2 market (Table 5). The load of contaminating germs is much higher than the standards prescribed in all markets. These microflora found in millet flour in Daloa have also been isolated in other cereal flours, notably corn flours in Abidjan city. Maize flour was contaminated with aerobic mesophilic microflora and loads ranged from 6.50 to 10.8 log CFU/g [22]. Some authors [34] mentioned that cereals are prone to a contamination and deterioration with moulds, yeasts, bacterial pathogens, coliforms, and Enterococci. These microorganisms must certainly have contaminated the millet grains during their storage after harvest and they were therefore naturally found in the by-product which is flour. These contaminants microorganisms could also originate from the utensils, the raw material (millet grains), the air or the water used to prepare millet flour. This author [35] has indeed indicated that such elements (water, utensils, etc.) are major sources of contamination of food products. The main source of these undesirable bacteria has been reported to be the grinding process and minor numbers of them could have also been introduced during unhygienic handling of the flour or the dough de cereals. Similar observations were reported by Wacher [36] by determining sources of microorganisms in *pozol*, a traditional Mexican fermented maize dough. The aerobic mesophilic microflora include mesophilic, pathogens and non-pathogens microorganisms [37] for the most part with low nutritional requirements. In addition, storage and sale conditions with little regard for hygiene rules and good manufacturing practices explain these high load. Furthermore, it has been shown that enterococci and coliforms are considered as hygiene indicators in the manufacturing process of foods [38]. The strong presence of these germs is justified by the fact that these microorganisms are ubiquitous. These bacteria, which are widespread in the environment and saprophytic in humans and warm-blooded animals, are found in flour during processing. Indeed, the process for transforming these flours is still traditional. The sufficiently long production time (at least 48 h) and inadequate hygienic conditions could constitute routes of contamination of these products. [39,40] showed in their study on the characterization of the traditional technology of flour production (based on corn) that the milling was a critical step, dependent on the amount of sunshine and the level of sanitation of the premises. Therefore, to avoid food borne illnesses due to enterococci and coliforms, cereal market flours must be prepared with good manufacturing practices and good conditions of storage. The high microbial count detected for yeast and moulds might be due to improper postharvest and storage handling of the cereal grains. The residue built up in traditional flour mills, as well as the wet process used can be relevant as additional sources of microbial contamination of commercial samples [32,41]. The Yeast and moulds growth on processed foods may lead to the formation of mycotoxins, which are secondary fungal toxic metabolites to humans and animals, causing disorders like cancer, immune suppression or endocrine disruption [42]. Therefore, storage conditions as relative humidity of atmosphere must be a critical control points during processing of cereal flours. In addition, these flours were contaminated with different potential pathogens with values ranging from 2.13 ± 0.01 log CFU/g to 6.08 ± 0.08 log CFU/g for *Escherichia coli*, from 7.18 ± 0.06 log CFU/g to 10.25 ± 0.16 log CFU/g for *Bacillus cereus* and from 3.32 ± 0.17 to 5.67 ± 0.66 log CFU/g for *Staphylococcus aureus* (Table 6). Whatever the study markets, the load in CFU / g of these germs is much higher than the prescribed standards. Similar results were found by [21,43] in infant flours produced in an artisanal way. The presence of these germs reflects a health risk for the consumer. Certain strains of *E. coli* are pathogens (diarrhea) that are usually found in environments with poor sanitary conditions [44].These results are consistent with those of [45] which showed that evaluation of the microbiological quality of *gappal* (a fermented food made from millet and milk) indicates the presence of *Bacillus cereus*, *Staphylococcus aureus* and *Enterobacteria* at levels close to or above the acceptable microbiological limits. Despite the advantages associated with fermentation, certain microbiological risks exist due to the fact that fermentation is natural in traditional processes. Pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella sp*., *Listeria sp*., *Campylobacter sp*., *Yersinia sp*. and *Brucella sp* have also been identified in fermented foods made from millet. Microorganisms in certain foods such as *gappal* have not been subjected to in-depth characterization and identification. Studies on the Lebanese *kishk* close to the *gappal* show that the bacteriological risk associated with this type of product is mainly linked to the sporulating flora characterized by the presence of *Bacillus cereus* and *Clostridium perfrigens* strongly represented in the samples [45].

5. Conclusion

The millet flour sale is a widespread activity in the markets of Daloa city. This study was carried out with a view to determining some physicochemical characteristics of millet flour sold on public markets in

Daloa city, but also to assess the microbiological risk associated with the consumption of this flour. Thus, the survey carried out made it possible to determine the socio-demographic profile of the millet flour sellers and also to detect the problems encountered during the sale of this flour. This survey revealed that the majority of the actors in this sector were women (100%). These women were illiterate (72.7%) and their age varied between 18 and 30 years (54.5%). The technical processing routes described by these millet flour vendors remained very empirical. The sorting of the millet grains is poorly done, the water used for hydration of the millet grains is not drinkable, the sieves are old and in poor condition and the machines used for processing the millet grains are archaic. The lack of good hygiene practices in places where millet flour is sold predispose it to all forms of microbial contamination. The pH of the millet flour collected from the different markets namely Lobia 2, Abattoir 2, Orly, Big market and Kennedy ranged from 4.55 ± 0.06 to 6.46 ± 0.42, and the majority of samples collected during this study had a pH below 5. Also, the moisture content of the millet flour taken from the different markets, was very high and varied from 17.33 ± 0.47 to 39.63 ± 0.09. Microbiological analyzes have shown that the millet flour sold in public markets in Daloa is highly contaminated with spoilage flora and other flora of fecal origin. The presence of pathogenic species such as coagulase positive *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* was noted in all the flours sold to Daloa. However, the origin of the fecal contamination of millet flour is strictly of animal origin. The strong presence of these germs would reflect a lack of good manufacturing practice and hygiene of millet flour, which would represent a danger for consumers. The consumption of millet flour sold on public markets in Daloa would represent a danger for the consuming populations.

References

[1] Statista, Production de céréale en volume au niveau mondial de 2008/2009 à 2018/2019. (Consulté en 2020) https://fr.Statista.com/statistiques/570915/céréale-volume-production-monde, 2020.

[2] FAOSTAT. (2018). FAO.doc_12_Mars_2018_12h12.doc. www.fao.org/faostat/fr/

[3] Chemisquy M.A., Giussani L.M., Scataglini M.A., Kellogg E.A. & Morrone O. "Phylogenetic studies favour the unification of *Pennisetum*, *Cenchrus* and *Odontelytrum* (Poaceae): a combined nuclear, plastid and morphological analysis, and nomenclatural combinations in Cenchrus", Ann Bot (Oxford, UK) 106:107-130. 2010.

[4] Jauhar P.P., Rai K.N., Ozias-Akins P., Chen Z. & Hanna W.W.Genetic improvement of pearl millet for grain and forage production: cytogenetic manipulation and heterosis breeding. In: Singh R.J., Jauhar P.P. (eds) Genetic resources, chromosome engineering, and crop improvement, 2nd edn. CRC Press, Taylor & Francis Group, Boca Raton, 2006. 281-307.

[5] Gowda C.L.L. & Rai K..N, "Evolution of hybrid parents research. In: Gowda CLL, Rai KN, Freddy BVS, Sana KB (eds) Hybrid parents research at ICRISAT. ICRISAT Center", Patancheru, pp 1-10. 2006.

[6] Kadry A., Halilou H. & Karimou. I., "Culture du mil (*Pennisetum glaucum* L. R. Br.) et ses contraintes à la production: une revue, *International Journal of Biological and Chemical Sciences*" 13(1): 503-524. 2019.

[7] Bashir E.M.A, Ali A.M, Ismail M.I, Parzies H.K & Haussmann B.I.G, "Patterns of pearl millet genotype-by- environment interaction for yield performance and grain iron (Fe) and zinc (Zn) concentrations in Sudan". Field Crops Research 166: 82-91. 2014

[8] Kannan B., Senapathy S, Raj A.G.B, Chandra S, Muthiah A, Dhanapal A.P & Hash C.T. "Association analysis of SSR markers with phenology, grain and stover-yield related traits in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)". The Scientific World Journal, 14. 2014.

[9] Hamadou M., Soumana I., Chaibou M., Souleymane O. & Kindomihou V., "Potentialités fourragères du mil (*Pennisetun glaucum* (L.) R.Br). Revue de littérature. Journal of Animal & Plant Sciences", 34(2): 5424-5447. 2017.

[10] Amadou I, Gounga M.E, & Le G.W. "Millets: Nutritional composition, some health benefits and processing" - A Review. *Emir. Journal. Food Agriculture*. 25: 501- 508. 2013.

[11] Amané N. D., Assidjo N. E., M. A., Bohoussou K. & Cardot P., "Caractérisation physico-chimique d'une bière traditionnelle ouest africaine: le tchapalo". *African Journal Online.* 17 (2): 143-152. 2005.

[12] N'Goran-AW E.B.Z., Doudjo S., Sadat A., David, A.K. & Emmanuel A.N., "Évaluation des caractéristiques physico-chimiques et microbiologiques d'un beignet traditionnel à base de mil fermenté (*gnomy*) commercialise dans la ville de Yamoussoukro (Côte D'Ivoire)", *European Scientific Journal*, 13(9): 227-241. 2017.

[13] RGPH (Recensement Général de la Population et de l'Habitat). Résultats globaux, institut national de la statistique, 1-12. 2014,

[14] ZAH B.T., Impact de la migration sur la démographie en côte d'ivoire in Revue de géographie du laboratoire Leïdi, n°13, 2015. 284-330.

[15] Kouassi K.C., Kouassi K.A., Yao K.M., Kouassi A.G. & Koffi N.R., "Assessment of the Risk of Microbial Contamination of an Urban Crop in the City of Daloa (Côte d'Ivoire): Case of Lettuce (*Lactuca sativa L.*)", Journal of Food Research 8(3): 122-132. 2019.

[16] AOAC, Official methods of analysis 16th Ed. Association of official analytical chemists. Washington DC, USA, 1995.

[17] Kimaryo V.M., Massawe G.A., Olusapo N.A. & Holzapfel W.M., "The use of starter culture in the fermentation of cassava for the production of "Kivunde" a traditional Tanzanian food product", *International Journal of Food Microbiology*, 56. 179-190. 2000.

[18] Coulin P., Farah Z, Assanvo J, Spillman H & Puhan Z. "Characterization of the microflora of attiéké, a fermented cassava product during traditional small-scale production", International journal of food Microbiology, 106, 131-136. 2006.

[19] Borrego A.F & Romero P. "Study of microbiological pollution of malaga littoral area II, Relationship between fecal coliform and fecal streptococci, VIème journée études", Pollution Cannes, 561-569. 1982.

[20] Dieye B. M., Le financement de la production maraîchages : l'exemple de la zone de Potou (Sénégal) ; BIM, février N°15, 6p, 2006.

[21] Malete Y., Sessou P., Farougou S., Metohoue R. & So- hounhloue D., "Évaluation de la qualité hygiénique de Tchaamessibu, une pâte acide consommée à Natitingou au nord Bénin", Revue de *Microbiologie Industrielle, Sanitaire, et Environnementale.* 7: 228-244. 2013.

[22] N'Goran-AW E.B.Z., Doudjo S., Sadat A., David, A.K. & Emmanuel A.N., "Qualité microbiologique des farines de maïs aux marchés d'Abidjan (Côte D'Ivoire)", *European Scientific Journal*, 13(9). 476-482. 2018.

[23] Tchekessi C.C.K., Bokossa IY., Hounkpatin GJ.F., Banon J., Adigun N., Sachi P. & Agbangla C., "Etude socio-économique et technologique de fabrication des boulettes de céréales pour la production d'une boisson fermentée de type probiotique consommée au Bénin", *International Journal of Innovation and Applied Studies*, (9): 1323-1335. 2014.

[24] McFeeters R. F. Effects of fermentation on the nutritional properties of food, p. 423-446. In E. Karmas and R. S. Harris (ed.), Nutritional evaluation of food processing. Van Nostrand Reinhold Co., New York, 1988.

[25] Alli I., Food Quality Assurance: principles and practices.CRC Press LLC , New York, USA, 2004, 141p.

[26] Mugula J.K., Nko S.A.M., Narvhus J.A. & Sorhaug T., "Microbiological and fermentation characteristics of *Togwa*, a Tanzanian fermented food", *International Journal of Food Microbiology*, 80, 187-199. 2003.

[27] Sripriya G., Usha A. & Chandra T.C., "Changes in carbohydrates, free amino acid, organic acid, phytate and HCl-extractability of minerals during germination and fermentation of finger millet (*Elveusive coracana*)", Food Chemistry, 58. 345-350. 1997.

[28] Assohoun N. M. C., Djéni N. T., N'Guessan K. F., & Koussemon M., "Preliminary study on antimicrobial properties of lactic acid bacteria involved in the fermentation of corn dough during *Doklu* processing in Côte d'Ivoire". Food Global Science Books, 6: 65-70. 2012.

[29] Zhang D.D., Liu J.L., Jiang T.M., Li L., Fang G.Z., Liu Y.P. & Chen L.J. "Influence of *Kluyveromyces marxianus* on proteins, peptides, and amino acids in Lactobacillus-fermented milk", *Food Sciences Biotechnology*, 26: 739-748. 2017.

[30] Nche P.F., Nout, M.J.R. & Rombouts F.M., "The effect of cowpea supplementation on the quality of *Kenkey*, a traditional Ghanaian fermented food". *Journal of Cereal Science*, 19. 191-197. 1994.

[31] FAO (Food and Agriculture Organisation of the United Nations), Codex alimentarius, Rome, 7: 1-54, 1994.

[32] Berghofer L.K., Hocking A.D., Miskelly D. & Jansson E., "Microbiology of wheat and flour milling in Australia", *International Journal of Food Microbiology*, 85:137-49. 2003.

[33] Ndjouenkeu R., Mbofung C.M.F. & Etoa F. X., "Étude comparative de quelques techniques de transformation du maïs en farine dans l'Adamaoua in Céréales en régions chaudes : conservation et transformation", Paris, France 1989, AUPELF/UREF, Edit. John Libbey Eurotext, pp. 179-186, 1989.

[34] Bullerman L. B. & Bianchini A., Food Safety Issues and The Microbiology of Cereals and Cereal Products. In book: Microbiologically Safe Foods, 2009, pp.315-335.

[35] Guiraud J.P., Microbiologie alimentaire. Edition DUNOD, Paris, 1998, 615 p.

[36] Wacher, M. C., Canas, A., Cook, P. E., Barzana, E., & Owens, J. D., "Sources of microorganisms in pozol, a traditional Mexican fermented maize dough", *World Journal of Microbiology and Biotechnology*, 9. 226-274. 1993.

[37] N'guessan Y.D., Bedikou M.E., Zoue L.T., Goualie B.G. & Niamke S.L., "Physicochemical, nutritive and safety evaluation of local cereal flours sold in areas of the District of Abidjan-Côte d'Ivoire", *Journal of Applied Biosciences*, 83: 7579-7594. 2014.

[38] Birollo G.A., Reinheimer J.A. & Vinderola C.G. "Enterococci vs. non lactic acid microflora as hygiene indicators for sweetened yoghurt", *Food Microbiology*, 18:597-604. 2001.

[39] Adjilé N., Houssou A.P.F., Monteiro N., Fainou M.C., Akissoe N.H. & Toukourou F. Caractérisation du procédé de gambari-lifin (farine de maïs décortiqué dégermé) et influence de la variété de maïs sur la qualité physicochimique et rhéologique. Nature & Technologie. B- Sciences Agronoaamiques et Biologiques, 2015, N° 12.

[40] Houssou P.A.F., Padonou S.W., Vodouhe M.C., Djivoh H., Dansou V., hotegni A.B. & Metohoue R., "Production du gambari-lifin (farine raffinée de maïs) de bonne qualité par l'amélioration du procédé traditionnel production au Bénin", *International Journal of Innovation and Applied Studies*, 17: 100-111. 2016.

[41] Shobha D, Prasanna Kumar MK, Putaramanaïk I & Sreematty TA., "Effect of antioxidant on the shelf life of quality protein maize flour", *Indian Journal of Fundamental Applied Life Sciences*, 1:129-140. 2011.

[42] Ntuli V., Mekibib S.B., Molebatsi N., Makotoko M., Chatanga P. & Asita. O.A., "Microbial and Physicochemical Characterization of Maize and Wheat Flour from a Milling Company, Lesotho", *International Journal of Food Safety*, 15: 11-19. 2013.

[43] Sanou A., Tapsoba F., Zongo C., Savadogo A. & Traore Y., "Étude de la qualité nutritionnelle et microbiologique des farines infantiles de quatre unités de production : CMA saint Camille de Nanoro, CSPS Saint Louis de Temnaore, CM saint Camille d'Ouagadougou et CHR de Koudougou", *Agronomic & Biological Sciences*, 17: 25-39. 2017.

[44] Mesa, R. J., V. Blanc, A. R. Blanch, P. Cortes, J. J. Gonzalez, S. Lavilla, E. Miro, M. Muniesa, M. Saco, M. T. Tortola, B. Mirelis, P. Coll, M. Llagostera, G. Prats, & F. Navarro, "Extended-spectrum beta-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage)", *Journal of Antimicrobial Chemother*, 58: 211-5. (2006).

[45] Tamime, T. Y. & Mcblulty D., "Kishk - a dried fermented milk/cereal mixture. Microbiological quality", *Lait* 79: 449-456. 1999.