Physicochemical Characteristics and Microbial Contamination of Raw Milk and Artisanal Milk Curd (Nonnon koumou) in Daloa, Côte D’Ivoire

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A B S T R A C T

Cow's milk is sought for its high concentration of animal protein and nutrients. Unfortunately, the conditions of milking, transport, packaging and marketing of milk in Daloa (Côte d'Ivoire) jeopardize its microbiological quality. Our study aimed to study the physicochemical and microbiological characteristics of raw milk (collected from pastures and sellers) and artisanal curd (nonnonkoumou). A total of 33 samples underwent physicochemical analysis. This analysis consisted of determination of pH, titratable acidity, and density. Microbiological analysis consisted of enumeration of lactic acid bacteria, yeasts and molds, Aerobic mesophiles, total and thermotolerant coliforms, and Salmonella research. A survey was also conducted to determine the knowledge, mode and frequency of milk consumption. This survey has shown that milk is known and consumed by many Ivorians and some nationals of neighboring countries of any age and any social level. The physicochemical analyses revealed a low pH (between 3.7 ± 0.007 and 3.9 ± 0.02) in the nonnonkoumou compared to that of the milk (between 5.9 ± 0.02 and 6.7 ± 0.007). Titratable acidity ranged from 1.4 ± 0.007 to 1.34 ± 0.007 in nonnonkoumou and from 0.36 ± 0.01 to 0.08 ± 0.007 in milk. Densities of milk and nonnonkoumou ranged from 0.841 ± 0.007 to 1.072 ± 0.02. Microbiological analyzes of milk samples taken from pastures showed similar loads (between 4.81 ± 0.4 and 6.88 ± 0.73 log CFU/ml) in fermentative germs and contaminants. In addition, the milk collected from the sellers was free of yeasts and molds and the total and thermotolerant coliforms were not detected in the nonnonkoumou samples analyzed. Salmonella (pathogenic flora) was detected in some milk samples analyzed thus demonstrating that this milk is of poor microbiological quality. Its consumption can lead to a food poisoning.

Keywords
Milk, nonnonkoumou, fermentation, lactic acid bacteria, contamination

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Introduction

Milk and dairy products contain many nutrients, such as protein, vitamins, calcium, phosphorus, magnesium, zinc, etc., which are necessary for healthful living of humans of all age groups and both sex (Das et al., 2015). As a concentrated source of macro- and micronutrients, milk and dairy products can play a particularly important role in human nutrition in developing countries, where the diets of poor people such as those in Côte d'Ivoire frequently lack diversity and consumption of animal source foods may be
limited (Muehlhoff, 2013). In Côte d’Ivoire, domestic production of artisanal milk is low and covers only 17% of overall milk consumption (Mirah-Dpp, 2012). To improve milk production, the country has set up, through development projects such as the Laitier Sud Project, small dairy farms mainly in peri-urban areas. To this end, small dairy farms are created in the peripheral areas of Abidjan town and other major cities (Daloa, Korogho.) of Côte d’Ivoire by officials or Ivorian or foreign businessmen who entrusted the management to the fulani from Mali, Burkina Faso or Guinea.

Thus, the largest quantity of milk produced is sold directly to consumers by breeders and small traders in informal, uncontrolled markets. (Youan-Bi, 2008).

The raw milk rapid degradation under the effect of the relatively high ambient temperatures characterizing tropical countries has forced actors in the "milk" sector to develop conservation or processing techniques adapted to the socio-economic and environmental context (Kèkè et al., 2009).

In Côte d’Ivoire, for example, cow's milk is transformed, apart from direct domestic consumption, into nonnonkoumou, a spontaneous fermented curd. The present fermentation of the artisanal milk is a spontaneous fermentation initiated by natural microorganisms that are found on raw materials, on the processing utensils/equipments, on the hands of producers and from the local atmosphere as natural starters (Jespersen et al., 1994). Despite its importance in the diet of large groups of people, the lack of control of fermentation process unavoidably results in significant variation in the quality and microbiological safety of this food (Vieira-Dalode et al., 2008). Milk and nonnonkoumou are subject to microbial contaminations and also to losses of their nutritional and market quality, due to limitations of traditional methods of production and conservation of these products.

Also, the unhygienic conditions of milking, transport, packaging and marketing of these perishable foodstuffs limit the provision to consumers of irreproachable quality products. So milk and dairy products can harbor a variety of organisms, including many zoonotic bacteria such as Brucella abortus, Brucella. melitensis, Campylobacter jejuni, Escherchia coli, Listeria monocytogens, Mycobacterium bovis, Mycobacterium tuberculosis, Salmonella, Staphylococcus aureus, and Yersinia enterocolitica, which can cause serious diseases, especially in children, pregnant women, elderly, and compromised individuals (Pal, 2007; FAO, 2013).

Furthermore diarrheal diseases, which include cholera and typhus as well as gastroenteritis, cause about 6 percent of all deaths throughout the world (Olshansky et al., 1997). To ensure a better microbiological quality of the raw cow's milk consumed by the Ivorian population, control of the process from the extraction of artisanal milk from pasture to commercialization would therefore be necessary. However, to our knowledge, no or few studies has been done on raw cow's milk extracted from pastures, as well as that of the sellers of Daloa town in Côte d'Ivoire.

The objective of this work was to study the physicochemical and microbiological characteristics of artisanal milk taken from pastures and from milk resellers and artisanal milk curd (nonnonkoumou) in Daloa. It will also be necessary to know the level of consumption of artisanal milk through a survey.
Materials and Methods

Survey of the consumption of artisanal milk

A preliminary survey allowed to identify pastures in Daloa town and to design a questionnaire of inquiry-consumption dedicated to milk consumers. The surveyed population consists of passers-by met on the main streets and side streets of five districts of Daloa town and volunteering to answer the questionnaire. In total, one hundred and fifty people were interviewed, with an average of about 30 people per ward. The survey consisted of a direct interview with the volunteers. This interview focused on the socio-demographic profile (age, study level...) of the consumer, his knowledge of milk, his mode and frequency of milk consumption and the appearance of possible symptoms of food poisoning (vomiting, diarrhea ...) related to the consumption of this food.

Sampling

Artisanal milk taken from four shepherds in pastures and from four (4) milk resellers and three (3) nonnonkoumou sellers were concerned by the sampling realized in Daloa town, Côte d’Ivoire. Three (03) samples of each product were collected by pasture and by seller. A total of thirty three (33) samples including twenty four for milk and nine for nonnonkoumou were collected in sterile containers and transported immediately in an icebox directly to the laboratory for analyses.

Physico-chemical analysis

The physico-chemical analysis was realized according to standard methods. pH values of the samples were measured by directly immersing the electrode of an electronic pH meter (Microprocessor pH meter, pH 211, HANNA Instruments) in 20 ml of milk. Total titratable acidity (TA) was measured by titrating 10 mL of milk against 0.1N sodium hydroxide (NaOH) solution using phenolphthalein as indicator (Kimaryo et al., 2000). To deduce the fraudulent practices of the sellers by adding external elements, the density of the milk samples of each seller was determined according to the formula using by Pointurier, 2003.

Enumeration of microorganisms

Preparation of stock solutions, inoculation of agar plates, and cultivation and quantification of microorganisms were carried out according to Coulin et al., 2006. For all determinations, 10 ml 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, enumeration of coliforms was carried out using VRBL (Violet crystal and neutral Red Bile Lactose) plates containing agar (VRBL agar, Oxoid Ltd., Basingstore, UK), incubated for 24 h at 30 °C for total coliforms and 44 °C for fecal coliforms. Yeasts and molds were enumerated on Sabouraud chloramphenicol agar (Fluka, Biochemica 89579, Sigma–Aldrich Chimie GmbH, India) incubated at 30 °C for 4 days. Aerobic mesophiles were enumerated on Plate Count Agar (PCA Oxoid.) and incubated at 30°C for 2 days. Enumeration of LAB was carried out using Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany), which were incubated under anaerobic conditions (Anaerocult A, Merck) at 37 °C for 72 h. Highlighting Salmonella sp is done in three stages (Pre-enrichment, enrichment and isolation) according to the reference standard NF/ISO 6579:2002 Amd 1: 2007. Pre-enrichment is performed in media Buffered Peptone Water (BPW) by incubating the stock solution at 37°C for 24 h. The enrichment consisted of taking 0.1 mL of the stock
solution (pre-enriched) and transferred to a tube containing 10 mL of Vassiliadis Rappaport broth previously prepared and sterilized. After homogenization, the tube is incubated at 42°C for 24 h. Finally the isolation was carried out from the enrichment medium incubated on a solid selective medium: Hecktoen agar. A drop is taken using a Pasteur pipette and then seeded by streaks on the surface of the Hecktoen agar. The dish is incubated at 37°C for 24 h, and sometimes even for 48 h, in the absence of characteristic colonies after the first incubation. On Hecktoen agar, the typical Salmonella colonies observed are green or blue with a black center.

**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.

**Results and Discussion**

**Milk consumption**

Of all the individuals interviewed during milk consumption survey in Daloa City, 53.33% were female and 44.66% were male. The age of these individuals ranged between 14 and 50 years or more with individuals majority (38.67%) in between the 25-50 age group. Among these individuals, 30% have no schooling level, 14% the primary level, the majority (37.33%) the upper level and the rest (18.67%) the secondary level. Most of them are pupils (16.67%), students (31.33%), civil servants (10.67%) and craftsmen (18.67). But, 9.33% of them do not exercise or more professional activity. These individuals were mostly single (57.33%) and most of them were Ivorian (84.67%). These individuals were mostly single (57.33%) and most of them were Ivorian (84.67%) (Table I).

The milk consumption survey has shown that milk is very well known and widely consumed. Indeed, 100% of the people interviewed knew this food well and consumed it all (100%) regularly and generally at breakfast time (45.33% of the individuals). The majority (61.33%) consumed it at least once a week, but others did it twice (14%) or even more (24.67%) a week. The budget for the consumption of milk could rise in some cases to more than 500 FCFA (for 15.33% of individuals) per day, but the majority (49.33%) spent between 100 and 200 FCFA per day to consume milk. Preference was given to boiled milk (40.67%), followed by fermented milk or nonnonkoumou (32%) and finally raw milk (27.33) (Table II).

According to the survey results, milk consumption was associated with disorders. Indeed, 46% of those questioned said they had had the ailments after drinking milk, compared with 54% of those who answered the opposite. The symptoms associated with these ailments were mainly diarrhea (89.85%) and gastroenteritis (10.15%). Thus most of the discomfort lasted on average 1 day (60.87%). However some lasted 2 days (30.43%) or more (8.70%). In addition, only 2.89% of the ailments mentioned required hospitalization compared to 97.10% (Table III).

**Change in pH, total titratable and density**

The pH of the milk samples taken from the pastures and from the sellers is relatively high compared to the pH of nonnonkoumou samples. Thus, the lowest pH is that of the
milk taken from pasture 3 (5.9 ± 0.02) while the pH of the milk taken from resellers 3 and 4 is highest with a value equal to 6.7 ± 0.007. These pH values are negatively correlated with the titratable acidity rate which varies from 0.36 ± 0.01 to 0.08 ± 0.02. On the other hand, nonnonkoumou samples have lower pH values ranging from 3.7 ± 0.001 to 3.9 ± 0.02.

The titratable acidity level of nonnonkoumou 2 and 3 is 1.4 ± 0.007 and that of nonnonkoumou 1 is 3.9 ± 0.02. The density of the milk collected in the pasture 4 and at the reseller 4 is equal to 1.029 ± 0.01. Also, the density of milk taken from the other 3 pastures and the other 3 resellers is between 1.04 ± 0.01 and 1.071 ± 0.01. The nonnonkoumou samples density varies from 0.832 to 0.860 (Table IV).

Enumeration of the different bacterial groups

Enumeration of microorganisms in this study showed that LAB and yeasts and molds had similar growth in the milk samples taken from pastures and their counts in this milk were not significantly different (P < 0.05) (Fig. 1). Indeed, the load of lactic acid bacteria (8.33 ± 0.5 log CFU / ml) is higher in the pasture milk 2 while that of yeasts and mold (6.88 ± 0.73 log CFU / ml) is higher in the pasture milk 1. The lowest loads of 6.23 ± 0.45 log CFU / ml for lactic acid bacteria and 5.10 ± 0.15 log CFU / ml for yeasts and molds are observed respectively in pasture milk 1 and 4 (Fig. 1).

The higher count of LAB was 5.94 ± 0.27 log CFU / ml in the reseller milk 2. The milk collected from reseller 3 contains less LAB with a load of 5.66 ± 0.45 log CFU / ml. In addition, the milk collected from the resellers was free of yeasts and molds (Fig. 2).

The yeast and mold loads in nonnonkoumou 1, 2 and 3 are substantially identical (from 5.94 ± 0.26 log CFU / ml to 6.12 ± 0.44 log CFU / ml) while the load in LAB are higher (5.52 ± 0.53 log CFU / ml) in nonnonkoumou 1 and lower (3.28 ± 0.46 log CFU / ml) in nonnonkoumou 3 (Fig. 3).

The pasture milk 4 contains the highest loads of aerobic mesophiles (6.53 ± 0.4 log CFU / ml), total coliforms (5.73 ± 0.42 log CFU / ml) and fecal coliforms (5.11 ± 0.38 log CFU / ml). On the other hand, the pasture milk 3 contains the lowest number of contaminating germs with loads of 4.98 ± 0.18 log CFU / ml for aerobic mesophiles, 4.89 ± 0.35 log CFU / ml for total coliforms and 4.81 ± 0.4 log CFU / ml for fecal coliforms (Fig. 4).

The aerobic mesophiles load is higher (6.36 ± 0.3 log CFU / ml) in the reseller milk 4 and lower (5.52 ± 0.41 log CFU / ml) in reseller milk 1. The number of total coliforms (5.74 ± 0.13 log CFU / ml) and thermotolerant coliforms (5.37 ± 0.28 log CFU / ml) was higher in reseller milk 2. Minimum loads are 5.06 ± 0.3 log CFU / ml for total coliforms and 4.98 ± 0.33 log CFU / ml for thermotolerant coliforms respectively in reseller milk 1 and reseller milk 4 (Fig. 5).

The Figure 6 shows the count of contaminating germs in nonnonkoumou samples. Indeed, the aerobic mesophiles loads in nonnonkoumou samples 1, 2 and 3 are substantially identical with values ranging from 6.68 ± 0.27 log CFU / ml to 6.86 ± 0.17 log CFU / ml. Moreover it is important to note that no coliforms (both total and thermotolerant) was detected in nonnonkoumou samples analyzed. Salmonella identification in milk and nonnonkoumou samples is presented in Table V. Thus, this table shows the presence of salmonella in the pastures milk 1 and 3 and in the reseller milk 1. Moreover it is important to note that no salmonella was detected in nonnonkoumou samples and in the other milk samples analyzed.
Table 1: Sociodemographic profile of individuals interviewed during the milk consumption survey conducted in the districts of Daloa city

| Sociodemographic profile of the individuals interviewed | Individuals number | Percentage (%) |
|----------------------------------------------------------|---------------------|----------------|
| Sex (n=150)                                              |                     |                |
| M                                                       | 83                  | 55.33          |
| F                                                       | 67                  | 44.66          |
| Age (n=150)                                              |                     |                |
| ≤ 14 years                                               | 04                  | 2.66           |
| 15-24 years                                              | 55                  | 36.67          |
| 25-50 years                                              | 58                  | 38.67          |
| > 50 years                                               | 33                  | 22             |
| Study level (n=150)                                      | No                  |                |
| Primary                                                  | 45                  | 30             |
| Secondary                                                | 21                  | 14             |
| Superior                                                 | 28                  | 18.67          |
| Other                                                    | 56                  | 37.33          |
| Occupation (n=150)                                       |                     |                |
| Pupil                                                    | 25                  | 16.67          |
| Student                                                  | 47                  | 31.33          |
| Civil servant                                            | 16                  | 10.67          |
| Artisan                                                  | 28                  | 18.67          |
| Unemployed                                               | 14                  | 9.33           |
| Other                                                    | 20                  | 13.33          |
| Marital status (n=150)                                   |                     |                |
| Married                                                  | 64                  | 42.67          |
| Single                                                   | 86                  | 57.33          |
| Nationality (n=150)                                      |                     |                |
| Ivorian                                                  | 127                 | 84.67          |
| Malian                                                   | 17                  | 11.33          |
| Burkinabe                                                | 05                  | 3.33           |
| Guinean                                                  | 01                  | 0.67           |

Fig. 1. Lactic acid bacteria and yeasts and molds (fermenting microorganisms) populations in milk samples taken from pastures. Values at each time point are the means of our replicates ± SD (error bars). LAB: Lactic acid bacteria, Y&M: Yeast and molds. PM1, PM2, PM3, PM4: Pasture milk 1, 2, 3 and 4. For the same type of germ, histograms bearing the same alphabetical letter are not statistically different (P > 0.05) (Tukey, HSD).
Table 2. Some aspects of milk consumption by the individuals interviewed during the consumption survey conducted in the districts of Daloa city.

| Raw milk Consumption aspects | Individuals number | Percentage (%) |
|-----------------------------|--------------------|----------------|
| Milk Knowledge (n=150)      |                    |                |
| Yes                         | 150                | 100            |
| No                          | 00                 | 0              |
| Milk consumption (n=150)    | Yes                |                |
| No                          | 150                | 100            |
| Day moments (n=150)         |                    |                |
| Breakfast                   | 68                 | 45.33          |
| Lunch                       | 21                 | 14             |
| Dinner                      | 37                 | 24.67          |
| Other                       | 24                 | 16             |
| Times number a day (n=150)  |                    |                |
| One time                    | 92                 | 61.33          |
| Two times                   | 21                 | 14             |
| Thrice                      | 00                 | 00             |
| Other                       | 37                 | 24.67          |
| The amount spent on buying milk by day (n=150) | | |
| 100-200 F                   | 74                 | 49.33          |
| 200-500 F                   | 53                 | 35.33          |
| 500 F+                      | 23                 | 15.33          |
| Types of milk consumed (n=150) |                |                |
| Raw                         | 41                 | 27.33          |
| boiled                      | 61                 | 40.67          |
| fermented                   | 48                 | 32             |

Fig. 2. Lactic acid bacteria and yeasts and molds (fermenting microorganisms) populations in milk samples taken from resellers. Values at each time point are the means of our replicates ± SD (error bars). LAB: Lactic acid bacteria, Y&M: Yeast and molds. RM1, RM2, RM3, RM4: Resellers milk 1, 2, 3 and 4. For the same type of germ, histograms bearing the same alphabetical letter are not statistically different (P> 0.05) (Tukey, HSD).
### Table 3: Consequences possibly linked to milk consumption by the individuals interviewed in the districts of Daloa city.

| Consequences linked to milk consumption | Individuals number | Percentage (%) |
|----------------------------------------|---------------------|----------------|
| Ailments after milk consumption (n=150) |                     |                |
| Yes                                    | 69                  | 46             |
| No                                     | 81                  | 54             |
| Symptoms of ailments (n=69)            |                     |                |
| Fever                                  | 00                  | 00             |
| Vomiting                               | 00                  | 00             |
| Diarrhea                               | 62                  | 89.85          |
| Boating                                | 00                  | 00             |
| Gastroenteritis                        | 7                   | 10.15          |
| Duration of ailments (n=69)            |                     |                |
| 1 days                                 | 42                  | 60.87          |
| 2 days                                 | 21                  | 30.43          |
| 3 days                                 | 00                  | 00             |
| other                                  | 06                  | 08.70          |
| Need for hospitalization (n=69)        |                     |                |
| Yes                                    | 02                  | 2.89           |
| No                                     | 67                  | 97.10          |

### Table 4: Acidity (pH and titratable acidity) and density of milk and *nonnonkoumou* samples.

| Parameters | pH          | Titratable acidity (%) | Density            |
|------------|-------------|-------------------------|--------------------|
| Samples    |             |                         |                    |
| PM1        | 6.1 ± 0.007\(^c\) | 0.23 ± 0.007\(^d\) | 1.060 ± 0.02\(^{ab}\) |
| PM2        | 6.2 ± 0.02\(^d\)  | 0.22 ± 0.01\(^d\)   | 1.071 ± 0.02\(^{ab}\) |
| PM3        | 5.9 ± 0.02\(^f\)  | 0.36 ± 0.01\(^c\)   | 1.040 ± 0.01\(^a\)  |
| PM4        | 6.5 ± 0.01\(^c\)  | 0.17 ± 0.007\(^s\)  | 1.029 ± 0.01\(^b\)  |
| RM1        | 6.5 ± 0.007\(^b\) | 0.06 ± 0.02\(^g\)   | 1.053 ± 0.02\(^{ab}\) |
| RM2        | 6.4 ± 0.01\(^c\)  | 0.08 ± 0.02\(^g\)   | 1.072 ± 0.02\(^{ab}\) |
| RM3        | 6.7 ± 0.007\(^a\) | 0.1 ± 0.01\(^f\)    | 1.045 ± 0.01\(^b\)  |
| RM4        | 6.7 ± 0.007\(^a\) | 0.08 ± 0.007\(^g\)  | 1.029 ± 0.01\(^b\)  |
| Nnk1       | 3.9 ± 0.02\(^g\)  | 1.34 ± 0.007\(^b\)  | 0.832 ± 0.02\(^c\)  |
| Nnk2       | 3.7 ± 0.001\(^h\) | 1.39 ± 0.02\(^a\)   | 0.860 ± 0.01\(^c\)  |
| Nnk3       | 3.7 ± 0.007\(^i\) | 1.4 ± 0.007\(^a\)   | 0.841 ± 0.007\(^c\) |

PM1, PM2, PM3, PM4: Pasture milk 1, 2, 3 and 4; RM1, RM2, RM3, RM4: reseller milk 1, 2, 3 and 4; Nnk1, Nnk2, Nnk3: *Nonnonkoumou* (artisanal milk curd) 1, 2 and 3. The same letter in the same column indicated no statistical difference (P> 0.05).
Table.5 Identification of Salmonella in milk and nonnonkoumou samples

| Samples | Germe          | Salmonella |
|---------|----------------|------------|
| PM1     | +              |            |
| PM2     | -              |            |
| PM3     | +              |            |
| PM4     | -              |            |
| RM1     | +              |            |
| RM2     | -              |            |
| RM3     | -              |            |
| RM4     | -              |            |
| Nnk1    | -              |            |
| Nnk2    | -              |            |
| Nnk3    | -              |            |

PM1, PM2, PM3, PM4: Pasture milk 1, 2, 3 and 4; RM1, RM2, RM3, RM4: reseller milk 1, 2, 3 and 4; Nnk1, Nnk2, Nnk3: Nonnonkoumou (artisanal milk curd) 1, 2 and 3.

Fig.3 Lactic acid bacteria and yeasts and molds (fermenting microorganisms) populations in nonnonkoumou samples taken from sellers. Values at each time point are the means of our replicates ± SD (error bars). LAB: Lactic acid bacteria, Y&M: Yeast and molds. Nnk1, Nnk2, Nnk3: Nonnonkoumou (artisanal milk curd) 1, 2 and 3. For the same type of germ, histograms bearing the same alphabetical letter are not statistically different (P> 0.05) (Tukey, HSD).
Fig. 4. Aerobic mesophiles, total and fecal coliforms populations (non fermenting microorganisms) in milk samples taken from pastures. Values at each time point are the means of our replicates ± SD (error bars). A.M.: Aerobic mesophiles, T. COL. Total coliforms, F. COL.: Fecal coliforms. PM1, PM2, PM3, PM4: Pasture milk 1, 2, 3 and 4. For the same type of germ, histograms bearing the same alphabetical letter are not statistically different (P > 0.05) (Tukey, HSD).

Fig. 5. Aerobic mesophiles, total and thermotolerant coliforms populations (non fermenting microorganisms) in milk samples taken from resellers. Values at each time point are the means of our replicates ± SD (error bars). A.M.: Aerobic mesophiles, T. COL. Total coliforms, F. COL.: Fecal coliforms. RM1, RM2, RM3, RM4: reseller milk 1, 2, 3 and 4. For the same type of germ, histograms bearing the same alphabetical letter are not statistically different (P > 0.05) (Tukey, HSD).
Fig. 6 Aerobic mesophiles, total and fecal coliforms populations (non fermenting microorganisms) in nonnonkoumou samples taken from sellers. Values at each time point are the means of our replicates ± SD (error bars). A.M.: Aerobic mesophiles, T. COL. Total coliforms, F. COL.: Fecal coliforms. Nnk1, Nnk2, Nnk3: Nonnonkoumou (artisanal milk curd) 1, 2 and 3. For the same type of germ, histograms bearing the same alphabetical letter are not statistically different (P > 0.05) (Tukey, HSD).

Raw cow’s milk is a staple food that is increasingly consumed by Côte d'Ivoire populations, particularly in Daloa town. The proof is that, during the survey on milk consumption, 100% of the people questioned (150) stated that they know raw cow's milk and consume it regularly (100%). They consume it at least once a day, preferably at breakfast (45.33%) and pay between 100 and 200 FCFA. These populations opted preferentially for boiled milk (40.67%), followed by fermented milk or nonnonkoumou (32%). In addition, some people surveyed (46%) reported having had ailments following the consumption of milk.

These ailments, most commonly characterized by diarrhea (89.85%) and gastroenteritis (10.15%), are usually attributed to the ingestion of a bad cooking food and / or having been subject to defective manipulations (Olshansky et al., 1997, Farthing, 2000). These conditions, marked by a high rate of diarrhea and gastroenteritis, are characteristic of the infections especially caused by sulphito-reducers Clostridium (Cassin et al., 1998). Cow's milk is consumed raw, boiled or fermented (nonnonkoumou). Indeed, the raw milk taken from the pastures and from the sellers has a pH varied from 5.9 ± 0.02 to 6.7 ± 0.007 relatively in conformity with the standards (IDF, 1995) which fixes the milk pH between 6.4 and 6.7.

On the other hand, the pH of nonnonkoumou samples is low (3.7 ± 0.001 to 3.9 ± 0.02) associated with titratable acidity levels ranging from 1.4 ± 0.007 to 1.34 ± 0.007. This considerable drop in pH in nonnonkoumou compared to milk is related to the fermentation that takes place during the manufacture of this food. Indeed, the decrease in pH suggests the presence and activity of LAB during the spontaneous fermentation of milk. LAB produce organic acids, mainly lactic and acetic acids, which induce a
lowering of pH (Coulin et al., 2006; Panda et al., 2007).

The rise in titratable acidity with the corresponding decline in pH during fermentation has been reported by several authors in studies on various African traditional foods such as mawe, sorghum beer and ogi (Hounhouigan et al., 1999; Sawadogo-Lingani et al., 2008; Oyarekua et al., 2008). Moreover, the density of the milk taken from the pasture 4 and from the reseller 4 is equal to 1.029 ± 0.01 and that of the milk taken from the other 3 pastures and the other 3 resellers varied from 1.045 ± 0.01 to 1.071 ± 0.01.

The nonnonkoumou samples density varied from 0.832 ± 0.02 to 0.860 ± 0.01. Thus, a density of less than 1.028 indicates the addition of water in the milk (wet milk) while a density greater than 1.032 indicates that the milk has been heated or skimmed. We can therefore deduce that the milk taken from the pasture 4 and that taken from the reseller 4 are normal milks.

These milks were not heated or added water. On the other hand, the milk of the 3 other pastures and the milk of the 3 other resellers having a density higher than 1.032 would have been heated before the commercialization. In addition, the density of nonnonkoumou below 1.028 could be explained by the addition of water to milk before or after the production of this curd.

The density of the milk also varies according to the dry matter content and the fat content. Thus it decreases with the increase of fat (Mahamedi, 2015). The microbiological parameters of milk and nonnonkoumou were also analyzed in this study. Indeed, the results obtained have shown the presence of LAB and yeasts and molds, in raw milk and in artisanal curd milk (nonnonkoumou). These microorganisms have had been previously isolated in some food and fermented food. These fermentative germs are abundant in the milk samples but especially in the nonnonkoumou samples and present a similar evolution.

The abundance of these microorganisms during fermentation is due to their tolerance to acidic conditions. They commonly play very important roles in the production of a range of traditional maize and cereal-based fermented foods and beverages, including koko (Lei & Jakobsen, 2004) and gowé (Vieira-Dalode et al., 2007) and have also previously been reported to be present in high numbers in fermented dough for doklu production (Assohoun et al., 2012). It has also been reported that LAB that survive the fermentation processes usually do this in association with yeasts (Sanni, 1993). Indeed, these microorganisms (yeasts and LAB) are common in traditional fermented foods (Nyanga et al., 2007).

A co-metabolism between these microorganisms has been suggested by Gobbetti et al., (1994). During this co-metabolism, LAB provide an acidic environment, which encourages the growth of yeasts, and yeasts provide vitamins and other growth factors for LAB. Yeasts would also make a useful contribution to the flavor and acceptability of fermented products according to Banigo et al., (1974). The variation in the concentration of lactic acid bacteria from one sample of nonnonkoumou to another may be explained by the fact that the technological processes used to manufacture this product differ from one producer to another. This variation may also depend on the initial milk composition of lactic acid bacteria and especially the storage conditions of this food (Ndiaye, 2002). These lactic acid bacteria produce several aromatic compounds, enzymes and other compounds that have a profound effect on the texture and taste of.
dairy products (Ngassam 2007). On the other hand, the milk collected from the resellers was free of yeasts and molds.

This absence of yeast and mold in our milk samples is probably due to the pasteurization of the milk before marketing. In fact, the milk taken from the sellers had a high temperature (around 70 ° C). Yeasts and molds are said to be heat-sensitive, that is, they do not tolerate high temperatures at all, hence the extermination of their population in these milk samples. Other microorganisms (total coliforms, fecal coliforms and aerobic mesophiles) were also present in samples analyzed in this study. Indeed, large GAM and coliform loads were detected in the different samples of milk and nonnonkoumou, and these charges varied from one pasture to another and from one reseller to another.

These contaminating germs certainly had to contaminate the milk during milking on pasture under generally unsanitary environmental conditions. Indeed, the surrounding air, the utensils used to extract milk and water could be the main sources of milk contamination. Guiraud (1998) has indeed indicated that such elements (water, utensils, etc.) are major sources of contamination of food products.

Also, the non-compliance with the rules of hygiene by the sellers, the transport conditions of milk of the pastures to the sale places, the dust as well as the flies which arise on the ladle serving as a measure during the sale, could also be contamination sources. Millogo et al., (2018) have demonstrated that containers used during milking and in the manufacture of fermented milk can cause contamination of the milk as well as the final product. In addition, Ndiaye in 2002 stated that the milk pH is favorable for microorganisms development, but their number decreased as the fermented milk aged. In addition, total and thermotolerant coliforms were detected in large number in all milk samples collected from pastures and resellers.

The incidence of coliforms in raw milk has received considerable attention, partly due to their association with contamination of fecal origin and the consequent risk of more pathogenic fecal organisms being present, partly because of the spoilage their growth in milk at ambient temperatures can produce. The presence of coliforms in dairy products is not acceptable by safe food consumption standards. These organisms are highly pathogenic and may cause serious diseases for human.

Coliform counts regularly in excess of 2 log are considered as evidence of unsatisfactory production hygiene (Directive 92/46/EEC, 1992). Sporadic high coliform counts may also be a consequence of unrecognized coliform mastitis, mostly caused by *E. coli* (Torkar et al., 2008). On the other hand, the nonnonkoumou samples analyzed were free of coliforms. This phenomenon is certainly due to the acidity of this food that has a low pH (below 4). This low pH prevents the growth of most spoilage and pathogenic organisms (Varga, 2007).

Such results have already been reported by some authors (Nout et al., 1989, Marsha et al., 1998, Karamoko et al., 2012) in their work on fermented foods. Also Steinkraus (1996) specified that coliforms do not tolerate low pH. This disappearing of coliforms was certainly due to the sensitivity of these bacteria to the substances produced by LAB. It was proved that LAB exert antimicrobial action through the production of lactic and acetic acids, bacteriocins, diacetyl and hydrogen peroxide (Daeschel, 1989). In addition, the salmonella presence was observed in the milk samples from pasture 1 and 3 and also from the reseller 3. The presence of salmonella in the milk of pasture 1 (PM1) and 3 (PM3) could be related to
contamination of the cows by this germ that has surely contaminated the milk. This presence may also be due to non-compliance with the rules of hygiene by the shepherds of pastures 1 and 3 during milking.

The presence of pathogenic bacterial species such as *S. aureus*, *E. coli* and *Salmonella sp* is a real danger for consumers of this food (Kouassi *et al.*, 2018). The presence of *Salmonella* in the milk collected from seller 3 may be associated with poor pasteurization of contaminated milk on pasture or during transport. It may also be due to recontamination of the milk after pasteurization due to poor hygienic practices.

On the other hand, *Salmonella* were not detected in *nonnonkoumou* samples, other pastures and milk other vendors. Their absence in the *nonnonkoumou* would be related to the acidity of the product which could inhibit the salmonella growth. These results are in agreement with those of Elham *et al.*, (2011) who found that all their samples of fermented dairy products were found free of Salmonella in Liban. These samples also comply with the standard provided by AFNOR in 1999 (fermented milks are free of *Salmonella*).

The main objective of this study was to study the physicochemical and microbiological parameters of milk collected from pastures, from women sellers and also from producers of *nonnonkoumou* in the Daloa city. A survey conducted to determine the knowledge, mode and frequency of milk consumption has showed that the milk is known and consumed by many Ivorians and some nationals of neighboring countries of all age and any social level. Milk consumption was associated with pathologies and the symptoms of food poisoning observed required very little medical intervention. The results of the physicochemical analyzes showed that the pH of the milk taken from the pastures and from the resellers was relatively high compared to the pH of the *nonnonkoumou*.

These results have also shown that some milk samples are normal while some have been pasteurized before marketing or have been water added. Moreover, the results of the microbiological analyzes showed the total disappearance of the yeasts and molds in the milk taken from the sellers and also an absence of total and thermotolerant coliforms in the *nonnonkoumou* samples analyzed.

These results also showed the absence of Salmonella in the majority of samples analyzed. On the other hand, the milk of pastures 1 and 3, as well as that of seller 1, contained this germ. The presence of Salmonella which is a pathogenic flora in these samples demonstrates that this milk is a poor microbiological quality. Its consumption can automatically lead to food poisoning.

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