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Microbiological Quality of Raw and Pasteurized Milk from the Korhogo Dairy

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Abstract The aim of this study is to make assessment of the hygienic and sanitary qualities of the raw and pasteurized milk sold by dairy of korhogo. To do this, 250 ml of raw and pasteurized milk were aseptically sampled from this dairy. The analyzes were targeted on hygiene quality indicator germs (Total Mesophilic Aerobic Flora, total coliforms, fecal coliforms, yeasts and molds) and pathogenic germs (Staphylococcus aureus and Samonella). Raw milk was loaded with mesophilic aerobic flora (3.16×10^5 CFU/ml), total coliforms (1.20×10^4 CFU/ml), faecal coliforms (8.10^3 CFU/ml), Staphylococcus aureus (1.56×10^4 CFU/ml) and yeasts and moulds (2.27×10^4 CFU/ml). Its hygienic and sanitary qualities were unsatisfactory. No salmonella colonies have been counted there. Pasteurized milk was also loaded with mesophilic aerobic flora (1.05×10^5 CFU/ml), total coliforms (5.88×10^3 CFU/ml) and fecal coliforms (8.73×10^2 CFU/ml). On the other hand, no colonies of Staphylococcus aureus, salmonella, yeasts or moulds were found in pasteurized milk. Its hygienic quality was unsatisfactory, but its sanitary quality was satisfactory. These results suggest that raw milk represents a health risk for consumers. In addition, it appears necessary to limit hygienic-fecal contamination of raw milk in order to obtain pasteurized milk of good hygienic quality.

Keywords: *korhogo, raw milk, pasteurized milk, hygienic quality, sanitary quality*

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1. Introduction

Milk is a major component of our nutrition and occupies a strategic place in our diet as it is an important balanced source of basic nutrients (proteins, carbohydrates and lipids), vitamins and minerals [1,2]. In West Africa, milk is a locally available nutritious product that plays an important role in the rural economy and in the health of a growing number of children [3]. It is an important part of the diets of pastoral or agro-pastoral families and urban consumers [4]. In Côte d'Ivoire, dairy production remains a secondary activity for the ivorian economy and is mainly dominated by traditional production. This production is estimated at around 34,000 tonnes [5]. It comes from traditional and semi-improved livestock farming in villages, urban and peri-urban areas. Traditional livestock farms are most often located around large cities and in the North of the country, which is home to 70% of the cattle population [6]. Korhogo, located in the north of Côte d'Ivoire, is an area of high cow's milk production. As a result, it houses a dairy which provides raw milk and pasteurized milk to meet the needs of the local population. Raw milk is normal mammary secretion of milking animals obtained from one or more milkings without addition to it or extraction from it, intended for consumption as liquid milk or for further processing [7]. As a result, several pathogens can be transmitted by raw milk, which is a vulnerable product [8]. In order to sanitize milk and meet the needs of the population under safe conditions, raw milk is generally subjected to heat treatments such as pasteurization, appertization and ultra-high temperature (UHT) sterilization. Pasteurization involves heating raw milk to 72°C for 15-20 seconds to destroy pathogens, while preserving its organoleptic quality and nutrients [9]. However, pasteurized milk can sometimes deteriorate and become unfit for consumption due to a number of factors, such as its residual microbial load, physicochemical composition, packaging method, storage temperature and duration, and the environment in which it is stored. These can reduce the nutritional value, as well as the microbiological and physicochemical qualities of the product [10]. Thus, microbiological control of milk before and after pasteurization is essential to ensure the safety of the milk made available to consumers. This study aims to evaluate the hygienic and health quality of raw milk and pasteurized milk marketed by the dairy in the city of Korhogo.

2. Materials and Methods

2.1. Biological Material

The biological material consisted of raw and pasteurized milk collected from the Korhogo dairy. The raw milk used by this dairy is supplied each morning by breeders from various farms in the Korhogo sub-prefecture. The collected milk is transferred into a container then divided into two parts. Part of the milk is sold raw and the other part is pasteurized before being marketed.

2.2. Milk Sample Collection

The milk samples were taken on three successive days, each morning after the dairy had been supplied with raw milk by the farmers. First, a quantity of raw milk ready for processing was homogenized, then 250 ml of sample was taken from it using a sterile 50 ml volumetric pipette and placed in a hermetically sealed sterile Stomacher bag (Figure 1). Then, after pasteurization at 90°C for 5 min, and cooling, 250 ml of pasteurized milk was drawn off in the same way as before (Figure 2). All these operations were carried out aseptically, and two types of milk (raw and pasteurized) were collected. Finally, the samples were placed in a cooler and transported to the Pelefero GON COULIBALY University laboratory for microbiological analysis. To avoid spoiling the milk, the time between collection and initial analysis was limited to 24 hours.

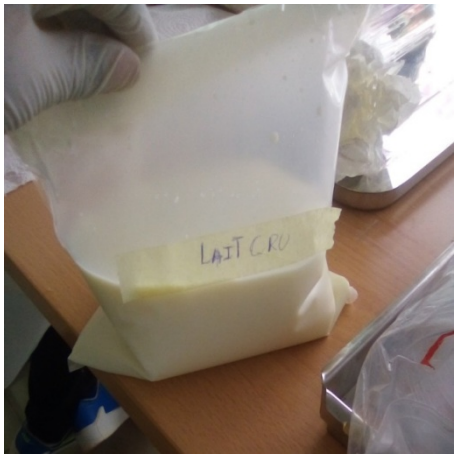

Figure 1. Raw milk

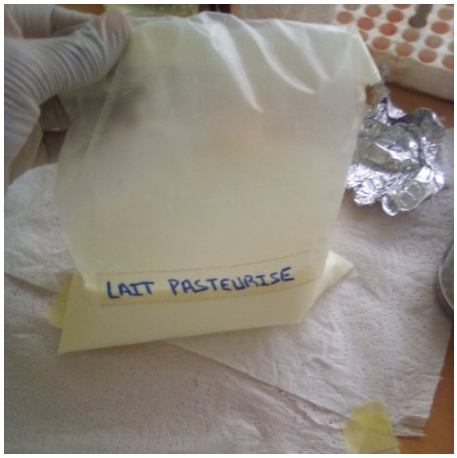

Figure 2. Pasteurized milk

2.3. Microbiological Analysis

Microbiological analysis of milk samples was carried out in accordance with the Ivorian standard (NI) proposed by CODINORM. The stock suspensions and the decimal dilutions were prepared according to standard NI 193 [11]. Total Aerobic Mesophilic Flora (TAMF) was enumerated on Plate Count Agar (PCA) according to standard NI 25 [12]. Total and faecal coliform counts were carried out on VRBL agar in accordance with standard NI 328 [13]. Yeasts and molds were tested on Sabouraud + Chloramphenicol Baird medium in accordance with standard NI 203 [14]. *Staphylococcus aureus* was counted on Baird-Parker agar with egg yolk and potassium tellurite according to standard NI 329 [15]. The count of each microorganism, expressed in CFU/ml, was carried out by counting the colonies characteristic of the microorganism sought according to standard NI 196 [16]. Salmonella testing was carried out according to standard NI 330 [17] in three successive stages, namely pre-enrichment, enrichment and inoculation.

Bacterial loads were compared with the tolerable threshold values in milk defined by ISO and AFNOR standards [18,19].

2.4. Statistical Processing of Data

The results of the microbiological analysis of the two types of milk were presented by the mean followed by the corresponding standard deviation, using EXCEL 2013 software. Student's paired-samples test was used to compare the microbial loads of raw milk with those of pasteurized milk. Statistical significance was set at $p < 0.05$. The test was performed with STATISTICA 2007.

3. Results and Discussion

3.1. Results

The application of microbiological criteria to the results of the various microbiological analyses carried out enabled us to assess the hygienic and sanitary qualities of the milk samples in this study.

3.1.1. Microbiological Quality of Raw Milk

3.1.1.1. Hygienic Quality of Raw Milk

Table 1 shows the results of the assessment of the hygienic quality of raw milk. All germs indicative of hygienic quality were present in raw milk. In addition, the average loads of FAMT ($3.16.10^5 \pm 1.40.10^4$ CFU/ml), total coliforms ($1.20.10^4 \pm 3.10^2$ CFU/ml) and fecal ($8.10^3 \pm 3.65.10^2$ CFU/ml), and yeasts and molds ($2.27.10^4 \pm 1.27.10^3$ CFU/ml) were higher than the tolerable threshold value. It follows from these results that the hygienic quality of the raw milk analyzed is unsatisfactory.

3.1.1.2. Sanitary Quality of Raw Milk

The results of the raw milk sanitary quality assessment are shown in Table 2. They showed an absence of salmonella, but an average load of *Staphylococcus aureus* ($1.56\times10^4 \pm 7.23\times10^2$ UCF/ml) which significantly

exceeded the maximum tolerated value. This last observation reflects the unsatisfactory sanitary quality of the raw milk analyzed**.**

3.1.2. Microbiological Quality of Pasteurized Milk

3.1.2.1. Hygienic Quality of Pasteurized Milk

Table 3 summarizes the results for the hygienic quality of pasteurized milk. With the exception of yeasts and molds, all the germs indicative of hygienic quality were present in pasteurized milk. The average loads of TMAF (1.05×10^5 ± 4×10^3 CFU/ml) and faecal coliforms (8.73×10^2 ± 4.18×10^1 CFU/ml) were within the acceptable range. However, total coliforms (5.88×10^3 ± 1.48×10^3 CFU/ml) were above the maximum acceptable limit. As a result, the hygienic quality of the pasteurized milk analyzed was unsatisfactory.

Table 1. Assessment of the hygienic quality of raw milk

Germs	Load (CFU/ml)	Limit tolerable value respect with ISO
TMAF *	3.16×10^5 ± 1.40.10^4	5×10^4
Total coliforms	1.2×10^4 ± 3×10^2	10^2
Faecal coliforms	8×10^3 ± 3.65×10^2	10^2
Yeast and moulds	2.27×10^4 ± 1.27×10^3	1.20×10^3

*TMAF stands for Total Mesophilic Aerobic Flora

Table 2. Assessment of the sanitary quality of raw milk

Germs	Load (CFU/ml)	Limit tolerable value respect with ISO
Staphylococcus aureus	1.56×10^4 ± 7.23×10^2	5×10^2
Salmonella	Absence	Absence in 25 ml

Table 3. Assessment of the hygienic quality of pasteurized milk

Germs	Load (CFU/ml)	Limit tolerable value respect with ISO
TMAF *	1.05×10^5 ± 4.10^3	5×10^4
Total coliforms	5.88×10^3 ± 1.48×10^3	10^2
Faecal coliforms	8.73×10^2 ± 4.18×10^1	10^2
Yeast and moulds	00 ± 00	1.20×10^3

*TMAF stands for Total Mesophilic Aerobic Flora

3.1.2.2. Sanitary Quality of Pasteurized Milk

The sanitary quality results for pasteurized milk are given in Table 4. They revealed an absence of *Staphylococcus aureus* and Salmonella. The sanitary quality of pasteurized milk is therefore satisfactory.

3.1.3. Comparison of the Microbiological Qualities of the Two Types of Milk Analysed

At the level of germs indicators of hygienic quality, the average microbial loads of germs tested in raw milk were very significantly ($p < 0.01$) higher than those quantified in pasteurized milk (Table 5).

As for those indicating sanitary quality, an absence of Salmonella was observed in both types of milk analyzed. However, the average load of *Staphylococcus aureus* in raw milk was highly significantly ($p < 0.001$) higher than that found in pasteurized milk, marked by an absence of this germ (Table 6).

These observations suggest that, of the two types of milk analyzed, pasteurized milk had the best hygienic and sanitary qualities.

Table 4. Assessment of the sanitary quality of pasteurized milk

Germs	Load (CFU/ml)	Limit tolerable value respect with ISO
Staphylococcus aureus	00 ± 00	5×10^2
Salmonella	Absence	Absence in 25 ml

Table 5. Comparison of the two types of milk with respect to germs indicative of hygienic quality

Germs	Milk types		Student's test
	Raw milk	Pasteurized milk	*P*-value (α = 0.05)
TMAF * (CFU/ml)	3.16×10^5 ± 1.40×10^4	1.05×10^5 ± 4.10^3	0.0011
Total coliforms (CFU/ml)	1.2×10^4 ± 3×10^4	5.88×10^3 ± 1.48×10^3	0.0182
Faecal coliforms (CFU/ml)	8×10^3 ± 3.65×10^2	8.73×10^2 ± 4.18×10^1	0.0007
Yeast and moulds CFU/ml)	2.27×10^4 ± 1.27×10^3	00 ± 00	0.0010

*TMAF stands for Total Mesophilic Aerobic Flora

Table 6. Comparison of the two types of milk with regard to germs indicating sanitary quality

Germs	Milk types		Student's test
	Raw milk	Pasteurized milk	*P*-value (α = 0.05)
Staphylococcus aureus (CFU/ml)	1.56×10^4 ± 7.23×10^2	00 ± 00	0.0007
Salmonella (CFU/ml)	Absence	Absence	ND *

* ND: Not determined

4. Discussion

The count of aerobic mesophilic microorganisms provides an indication of the degree of contamination of the food. Counting the total aerobic mesophilic flora of raw milk gave a microbial load of 3.16×10^5 ± 1.40×10^4 CFU/ml. This value is below the acceptable limit (5×10^4 CFU/ml). This result indicates that the raw milk was collected under good hygienic conditions, as the aerobic mesophilic flora generally provides information on the hygienic quality of raw milk. It is also considered to be the determining factor in the shelf life of fresh milk [20]. Total mesophilic flora is also a good indicator of overall product quality and stability [21]; [2]. It is the most sought-after flora in microbiological analyses. According to [22], the level of contamination depends on the hygienic conditions in which handling is carried out, i.e. the state of health of the animal, particularly the udder, and the level of contamination of the stalls, milking parlour, teats and milk collection equipment. These results differ from those of [23,24], and [25], who showed the widespread predominance of AFM (Aerobic Mesophilic Flora) on dairy farms in the Central, Central-Western and Southern regions of Côte d'Ivoire.

Total and faecal coliform counts in raw milk of 1.2×10^4 ± 3.10^2 CFU/ml and 8.10^3 ± 3.65×10^2 CFU/ml, respectively, indicate results above the standard (10^3

CFU/ml). According to [26], the abundance of faecal coliforms in raw milk indicates non-compliance with sanitary requirements during milking and harvesting. In fact, contamination can occur during faulty transport or storage. In addition, the presence of coliforms in certain samples is evidence of defective hygiene during processing, which may be due to the processor, the equipment in contact and/or the product's immediate environment. These bacteria are generally not dangerous from a health point of view, except in the case of extremely abundant proliferation or particular consumer sensitivity. In general, a microbial load of less than 100 CFU/g of product is tolerated. The results obtained are superior to those of [27], who obtained values of 6.8×10^{2} CFU/ml, for faecal coliforms, and 1.02×10^{2} CFU/ml, for total coliforms, in the analysis of raw cow's milk in Algeria. According to [28], the presence of these germs in milk can also be linked to contamination by cow dung, soil and water.

Analyses revealed a high presence of yeasts and moulds ($2.27\times10^{4}\pm 1.27\times10^{3}$ CFU/ml), well above the tolerable limit (1.20×10^{4} CFU/ml) in raw milk.

The results of this study are in line with those of [29], who also noted a high presence of yeasts and molds (4.4×10^{4} CFU/ml) in his analysis of raw milk in Mali.

These 6 germs - total aerobic mesophilic flora, faecal and total coliforms, yeasts and moulds - are not recognized as a danger to human health. Nevertheless, the samples concerned remain unsatisfactory for human consumption in terms of their hygienic quality.

With regard to germs indicating sanitary quality, significant contamination by *Staphylococcus aureus* ($1.56\times10^{4}\pm 7.23\times10^{2}$ CFU/ml) was noted, and no Salmonella was found in the raw milk. The considerable average load of *Staphylococcus aureus*, well above the recommended standard (5×10^{3} CFU/ml), indicates unsatisfactory sanitary quality of the raw milk. Staphylococci pose a real risk to public health in processed products, as they can produce heat-stable enterotoxins resistant to heat treatment under certain conditions [30]. According to [31] *Staphylococcus aureus* is considered a major pathogenic bacterium, causing mammary infections. These are accompanied by an increase in permeability between the blood compartment and the milk, resulting in changes in milk composition [32]. Mammary staphylococcal infections are the main source of milk contamination during production; other sources of contamination are also to be considered, such as the milking machine [33]. The load obtained for *Staphylococcus aureus* in this study is lower than that (5×10^{7} CFU/mL) obtained in Mali for this germ [34,35].

Microbiological analysis of pasteurized milk revealed that the loads of total aerobic mesophilic flora and faecal coliforms in pasteurized milk were $1.05\times10^{5}\pm 4\times10^{3}$ CFU/ml and $8.73\times10^{2}\pm 4.18\times10^{1}$ CFU/ml respectively, values below the [18]. However, these results are superior to those of [36] who had total germs in their analysis of pasteurized milk in Algeria with values varying between 9×10^{2} and 6×10^{3} CFU/ml. In addition, contamination levels of total aerobic mesophilic flora and total coliforms in raw and pasteurized milk varied significantly ($p<0.05$) during this study. In fact, pasteurized milk recorded the lowest loads. These loads are in line with the recommended standard. A comparison of the microbiological qualities of the two types of milk provides an insight into the effect of pasteurization on the hygienic and sanitary qualities of raw milk. These results suggest that pasteurization has improved the hygienic quality of raw milk. On the other hand, total coliform counts in pasteurized milk ($5.88\times10^{3}\pm 1.48\times10^{3}$ CFU/ml) were higher than the recommended standard (10^{3} CFU/ml). According to [27] the presence of high levels of faecal coliforms seems to be linked to several factors, such as poor staff hygiene, equipment and premises decontamination protocols, and poor milk storage or protection conditions. Packaging conditions are at the root of milk contamination after processing, i.e. during packaging or the use of poor-quality packaging materials. What's more, the equipment used for the various dairy manipulations presents a risk of contamination before pasteurization and recontamination after pasteurization, due to its regular contact with the raw material [37].

The total absence of yeasts and molds, Salmonella and *Staphylococcus aureus* in pasteurized milk complies with European Commission recommendations. The results obtained differ from those of [38], who reported the presence of Salmonella and *Staphylococcus aureus* in their sample. Thus, the absence of these germs would also testify to the improved sanitary quality of raw milk as a result of the heat treatment applied.

5. Conclusion

Our study revealed that raw milk does not meet microbiological criteria relating to faecal and total coliforms, yeasts and moulds, and *Staphylococcus aureus*. As a result, the hygienic and sanitary qualities of raw milk are unsatisfactory. Furthermore, although the hygienic quality of pasteurized milk is better than that of raw milk, it remains unsatisfactory due to the relatively high presence of total coliforms. However, pasteurized milk, which complies with standards for Salmonella and *Staphylococcus aureus*, has a good sanitary quality. This study needs to be taken further. For this, it would be desirable to apply microbiological analysis to milk samples representative of the study area and collected at different production periods. Their physicochemical and biochemical qualities should also be determined.

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