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Microbiological and Physicochemical Quality of Raw Milk Marketed in the City of Pala in Chad

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Abstract

Milk is a food with high nutritional value. Its importance results from its richness in water, proteins, lipids, minerals and vitamins. The objective of this study was to evaluate the physico-chemical and microbiological quality of raw cow milk marketed in Pala, Chad. A total of thirty (30) samples were collected. The standard microbiology technique was used to enumerate and test for total aerobic mesophilic flora (TAMF), *Escherichia coli*, *Staphylococcus*, yeasts and molds, and *Salmonella spp*. Dornic acidity content was also assessed. TAMF, *E.coli*, *Staphylococcus*, yeast & molds were dissatisfied with the quality in the proportions of 20%, 17%, 83.3% and 7% respectively. Salmonella was totally absent in all samples. The Dornic acidity was high in all samples with a value between 22 and 30 °D. In view of these results, raw milk has an acceptable hygienic quality despite the low proportions of dissatisfaction with the quality observed. Efforts to raise awareness of hygiene measures must be made in the milk sector in order to restore its expected safe quality.

Keywords: raw milk, microbiological quality, physicochemical quality, Pala, Chad

1. Introduction

The milk sector occupies an important place both in society and in the country's economy. According to the FAO (2018), the Chadian livestock numbers 93.8 million cattle (Aliba , 2019). Milk is the only nutritional source considered a complete and essential food (Iqbal *et al.*, 2016, Lucey, 2015). It is a food with high nutritional value. Its importance results from its richness in water, proteins, lipids, minerals as well as vitamins (Katinan *et al.*, 2012; Doutoum, 2022). This is how it meets the need for growth and maintenance of young babies and its consumption at all ages is a consensus in the scientific sphere (Vignola *et al.*, 2002; Fox, 2014). The populations of the arid and semi-arid regions of the globe practicing animal husbandry associated with agriculture have made milk a staple of their diet (Katinan *et al.*, 2012). In Chad, the consumption of milk and dairy products is also very popular and the need is growing with the population explosion. Depending on the environment and the possibility of conservation, milk can be consumed in different forms (Koussou *et al.*, 2007; Doutoum *et al.*, 2013). The use of raw milk refrigeration is not always a reliable way to overcome contamination problems, or promote better preservation (Aliba, 2019). However, its nutritional importance makes it favorable to the development of microorganisms, especially pathogenic and harmful to the health of the consumer. And also, the pathogenic flora that the milk can harbor will be all the more important when the milk is milked under questionable hygienic conditions (Koussou et al., 2007; Doutoum *et al.*, 2013; Ma ïwore *et al.*, 2018). Raw milk, generally sold at a low price in the town of Pala and especially in the rainy season, occupies a prominent place in the eating habits of the population. This foodstuff prized by the population of the city is produced by breeders with little or no education, thus milking in the open air in inadequate hygienic conditions. The production of milk and dairy products must

be rigorously controlled due to possible risks that may constitute a health problem for consumers (Aliba, 2019; Valery, 2016). In Chad, few studies have concerned the microbiological and hygienic quality of milk. Thus, the present study aimed to evaluate the physico-chemical and microbiological quality of raw milk marketed in the city of Pala knowing that it has not been the subject of any scientific study of the kind previously.

2. Material and Methods

2.1 Framework and Period of Study

The research work was carried out in the laboratory of the Food Quality Control Center (CECOQDA) in the Department of Food Microbiology located in N'Djamena in Chad from July to September 2022.

2.2 Prospective Survey

A prospective survey was conducted in the peri-urban area in the city of Pala, capital of the Mayo Kebbi -West region and also capital of the Mayo-Dallah department. The purpose of the survey is to obtain information on the education levels of breeders. In addition, it also targeted the working environment; milker hygiene; the materials used during milking; veterinary follow-up and transport. The survey was carried out on the basis of a form.

2.3 Sampling

The samples were taken in the city of Pala, capital of the Mayo Kebbi-Ouest region and also capital of the Mayo-Dallah department. The samples were collected using aseptic technique, to prevent the increase of the initial microbial load in the sampled product during collection. It was done randomly.

For this study, a total of thirty samples of raw milk were taken in sterile 300mL bottles from six different producers. Thus, five samples were taken from each producer. The samples were taken as they are sold to consumers and sent to the CECOQDA laboratory for analysis in a cooler fitted with dry ice. Samples were stored at 4 ℃. They were analyzed 24 hours after sampling.

2.4 Microbiological Analyzes

2.4.1 Sample Preparation

Raw milk sample, taken in sterile bottles, are homogenized and 25g are aseptically weighed and then mixed with 225mL of buffered peptone water (interscience balance gravimat). After mixing with a blender (Stomacher interscience), the decimal dilutions were made and we retained the dilutions 10 -1 to 10 -4 . These different dilutions retained were inoculated onto agar culture media and incubated (Binder KB53 oven) at temperatures corresponding to each germ sought.

The counting of the total aerobic mesophilic flora (FMAT) was carried out on PCA agar (Plate Count Agar) by inoculation in depth of 100 µL of the 10-1 to 10-4 dilutions . The incubation time was 37 ℃ for 18-24 hours. The result is read using a colony counter equipped

with a magnifying glass (Interscience Scan100).

The search for and enumeration of *Escherichia coli* was carried out on TBX medium (Tryptone Bile X- glucuronide) after 24 hours of incubation at 44 ℃. Colonies appearing blue or blue/green after absorption of the chromogen were considered. The search for and counting of staphylococci were carried out on Chapman's medium with mannitol and dilutions of 10^{-1} and 10^{-2} were used. A 0.1mL suspension of the inoculum was surface-seeded and incubated for 18-24 hours at 37 ℃. Staphylococcal colonies were confirmed by the catalase test in hydrogen peroxide (LABELL) and by Gram staining (bioMérieux reagents). The slides were read using a light microscope (Olympus) at the 100 objective with immersion oil. Catalase + and Gram + Cocci arranged in clusters or diplococci were considered.

The enumeration of yeasts and molds was carried out on OGA agar (Oxytetracycline Glucose Agar) after 72 hours of incubation at 25 ℃. Using the colony counter, average cream-colored, semi-domed colonies were identified for yeasts and filamentous colonies of varying colors depending on the type of mold.

The search for Salmonella was carried out in three stages:

- The pre-enrichment which was done by suspending 26 g of the sample in 125 mL of buffered peptone water. This broth is incubated at 37 °C. for 24 hours.
- The enrichment was carried out using pre-enriched cultures. 100 µL of pre-enrichment broth are inoculated onto Rappaport-Vassiliadis broth (RVS) and incubated at 37 ℃ for 24 hours for selective enrichment.
- Finally, the isolation, which is the last step, was carried out simultaneously on XLD (Xylose Lysine Deoxycholate) and Hektoen agar. A drop of culture on RVS was inoculated on XLD and another drop on Hektoen®, then left to incubate at 37 ℃ for 24 hours. This is followed by counting, which focused on colonies with a black center, and characterization on an API 20E Strep gallery (Biomerieux).
- A colony isolated from a pure culture was used to form a bacterial suspension in distilled water. The supports of the incubation box have been soaked by water to create a humid atmosphere, then the gallery is placed in the incubation box. Using a pipette, the compartments are filled with the previously prepared bacterial suspension. After incubation for 24 hours at 37 ℃, the reading of the positive or negative reactions is done according to the color variations.

2.4.2 Microbiological Standards

The chemical and microbiological standards allowing the interpretation of the results are presented in Table 1 below.

Table 1. Chemical and microbiological quality criteria for raw milk (Luxemburg, 2018)

Settings	Standards
Titratable acidity (°D)	14-18
TAMF	< $5x10^4$
E.coli	<10
Staphylococcus	102
Yeasts and molds	$5x10^4$
Salmonella spp.	Absence in 25g

TAMF: total aerobic mesophilic flora; *E.coli* : *Escherichia coli*, *Salmonella spp.* : potentially pathogenic salmonella strains.

2.5 Physico-chemical Analyzes: Measurement of Dornic Acidity

Acidity was measured by titration. To do so, 10mL of the sample was taken and poured into a beaker. Two to three drops of phenolphthalein were added to the milk and the mixture was homogenized using bars. The titration is measured at ambient temperature (25 °C) by adding Dornic soda (NaOH N/9) drop by drop, previously placed in a graduated burette, until the color changes to pale pink. The total volume of soda added was noted and multiplied by 10 for the expression of the results (AOAC, 2005). The acidity was expressed in °D (1 °D corresponds to 0.1 g of lactic acid per liter of milk). For each sample, the operation was repeated twice.

2.6 Statistical Analyzes

The data collected during this study was entered on the Excel 2013 workbook and transferred to SPSS version 26 for the calculation of averages.

3. Results

3.1 Results of Microbiological Analyzes

The results of the microbiological analyzes (expressed in CFU/ mL) are presented in tables 2, 3 and 4 and include all the parameters studied on the thirty samples. Each table thus presents ten samples.

Table 2. microbiological results of the first ten samples

Sample	TAMF	*E.coli*	Staph	Yeasts & Molds	*Salmonella*
E1	$1.9x10^4$	<10	$6x10^2$	90	Absence
E2	$2.2x10^3$	<10	$7x10^2$	96	Absence
E3	$1.2x10^4$	<10	$2x10^3$	88	Absence
E4	$5.5x10^5$	$1.1x10^2$	$1.6x10^5$	$2.1x10^3$	Absence
E5	$1.1x10^3$	<10	$1.3x10^3$	86	Absence
E6	$1.9x10^3$	<10	$5x10^2$	90	Absence
E 7	$2.7x10^5$	$5x10^1$	$1.3x10^4$	60	Absence
E8	$2x10^5$	$1.4x10^2$	$1.5x10^4$	80	Absence
E9	$8x10^3$	<10	$1.5x10^3$	78	Absence
E10	$2.4x10^5$	$6x10^1$	$1.5x10^5$	$4x10^2$	Absence

TAMF: Total Aerobic Mesophilic Flora; *E.coli* : *Escherichia coli* ; Staph : staphylococci; Salmonella spp : *Salmonella* potentially pathogenic strains. E: sample.

The first ten (10) raw milks examined in the city of Pala contain a variable load of TAMF, located between 1.1x10 ^{3} CFU / ml to 5.5x10 ^{5} CFU / ml. With regard to *E. coli*, their loads vary between 1.1x10 ^{2} CFU /ml at 1.4x10 ^{2} CFU /ml. The enumeration of *Staphylococcus aureus* gave a value of between 5x10 ^{2} CFU/ml to 1.6x10 ^{5} CFU/ml. Yeasts and molds vary between 4x10 ^{2} CFU /ml at 2.1x10 ^{3} CFU /ml.

Table 3. Microbiological results of the second ten samples

Sample	FMAT	*E.coli*	Staph	Yeasts & Molds	Salmonella
E11	6.4x10 ^{5}	4x10 ^{1}	9.7x10 ^{3}	5x10 ^{2}	Absence
E12	1.9x10 ^{4}	<10	4.3x10 ^{2}	76	Absence
E13	8.8x10 ^{3}	<10	6x10 ^{2}	92	Absence
E14	6.9x10 ^{5}	<10	2.2x10 ^{5}	20	Absence
E15	5.5x10 ^{4}	1x10 ^{4}	3.1x10 ^{5}	4x10 ^{2}	Absence
E16	1.7x10 ^{3}	<10	96	78	Absence
E17	1.6x10 ^{4}	<10	4.5x10 ^{3}	82	Absence
E18	1.3x10 ^{4}	<10	2x10 ^{5}	2.2x10 ^{3}	Absence
E19	7.7x10 ^{3}	<10	56	20	Absence
E20	2.1x10 ^{3}	<10	68	42	Absence

The second analysis of ten (10) samples of raw milk contains, the Aerobic Flora Total mesophilic at a variable load, between 2.1x10 ^{3} CFU/ mL to 6.9x10 ^{5} CFU/ mL. *E.coli* have a bacterial load varying between 4x10 ^{1} CFU/ mL to 1x10 ^{4} CFU/ mL . The enumeration of *Staphylococcus aureus* gave a value of between 4.3x10 ^{2} CFU/ mL to 3.1x10 ^{5} CFU/ mL. The yeasts and molds show a result varying from 5x10 ^{2} CFU/ mL to 2.2x10 ^{3} CFU/ mL.

Table 4. Microbiological results of the third ten samples

Sample	FMAT	*E.coli*	Staph	Yeasts & Molds	Salmonella
E21	1.2x10 ^{4}	<10	86	96	Absence
E22	1.2x10 ^{4}	<10	1.7x10 ^{3}	78	Absence
E23	7.3x10 ^{5}	<10	1.7x10 ^{5}	66	Absence
E24	1.3x10 ^{4}	<10	6x10 ^{2}	89	Absence
E25	3.1x10 ^{6}	8x10 ^{2}	1.1x10 ^{4}	8x10 ^{2}	Absence
E26	3.1x10 ^{6}	4.8x10 ^{2}	1.2x10 ^{5}	4x10 ^{2}	Absence
E27	1.0x10 ^{4}	4x10 ^{1}	3.1x10 ^{3}	86	Absence
E28	3.1x10 ^{3}	<10	86	94	Absence
E29	1.1x10 ^{4}	<10	7.2x10 ^{2}	97	Absence
E30	5x10 ^{4}	<10	1.0x10 ^{4}	4x10 ^{2}	Absence

The analysis of the third ten samples of raw milk contains a variable load of the FMAT, located between 3.1x10^{3} CFU/ mL to 3.1x10^{6} CFU/ mL. For *E. coli* , the bacterial load varies between 4x10^{1} CFU/ mL to 8x10^{2} CFU/ mL . The enumeration of *Staphylococcus aureus* gave a value of between 7.2x10^{2} CFU/ mL to 1.7x10^{5} CFU/ mL. Yeasts and molds are

between $4x10^2$ CFU/ mL to $8x10^2$ CFU/ mL.

Research of *Salmonella* is the last microbiological parameter considered in this study. The exploration of the three preceding tables of results shows that *Salmonella* are completely absent in all the thirty samples analyzed. *Salmonella* being the main causes of collective food poisoning and highly sought after germs in food microbiology, food intended for human consumption must be free of any *Salmonella* as recommended by the Luxemburg standard

3.2 Results of Microbiological Quality Regard to Pathogenic Germs

3.2.1 Microbiological Quality According to TAMF

The TAMF is an indicator of the microbiological quality of the food, it reflects the exposure of the sample to any contamination and in general the existence of conditions favorable to the growth of microorganisms.

Enumeration of this flora is useful to indicate whether cleaning and disinfection during milking have been sufficiently carried out. This concentration of TAMF does not necessarily indicate the internal contamination of the milk before milking or the sanitary state of the animal, because it is a general indicator of the state of hygiene. The raw milk samples taken in the city of Pala have variable thresholds according to the standard (Luxemburg, 2018) recommended at $<5x10^4$. This testifies to the application of good hygiene practices at the time of milking. It is 70% satisfactory, 10% acceptable and 20% unsatisfactory.

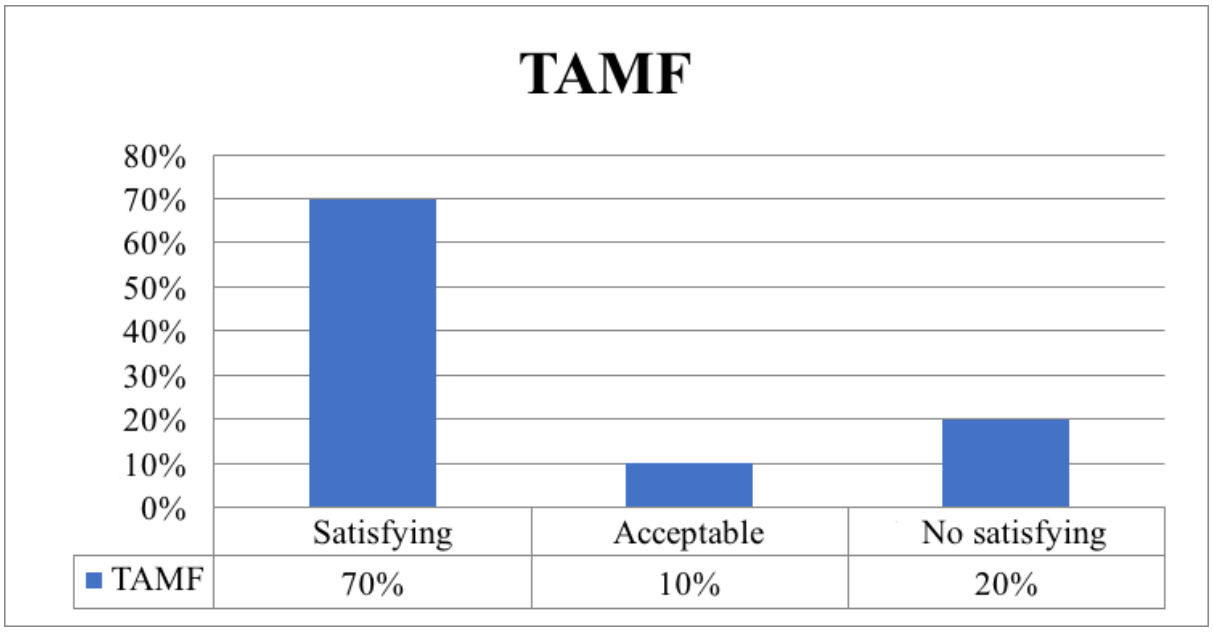

Figure 1. Microbiological quality according to hygiene indicator germs

3.2.2 Microbiological Quality According to Escherichia Coli

Escherichia coli is considered as evidence of faecal contamination of foodstuffs following handling.

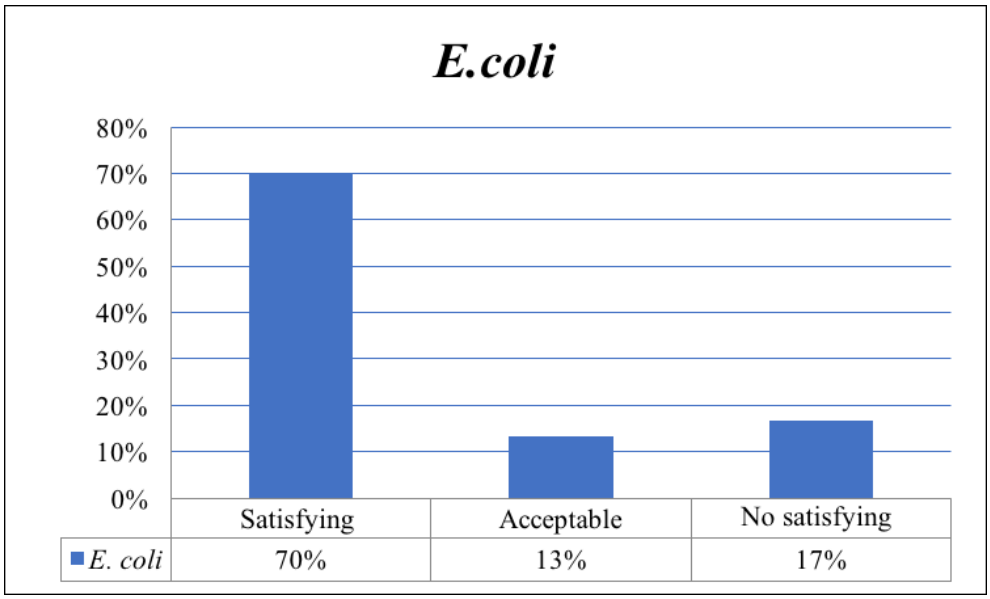

Figure 2. Microbiological quality according to *E.coli*

Escherichia coli are considered as evidence of faecal contamination of foodstuffs following handling. In accordance with the threshold of the set standard of colonies (<10) of Luxemburg, 2018, 70% of the samples of raw milk are rated satisfactory, 13% Acceptable and 17% are unsatisfactory.

3.2.3 Microbiological Quality According to Staphylococcus Aureus

Staphylococcus aureus is a contagious agent that lives on the udder of cows and is transmitted from cow to cow (Makovec et al ., 2003). This bacterium can gain access to milk either by direct excretion from udders with clinical or sub -clinical staphylococcal mastitis, or by environmental contamination during handling and processing of raw milk by dirty hands.

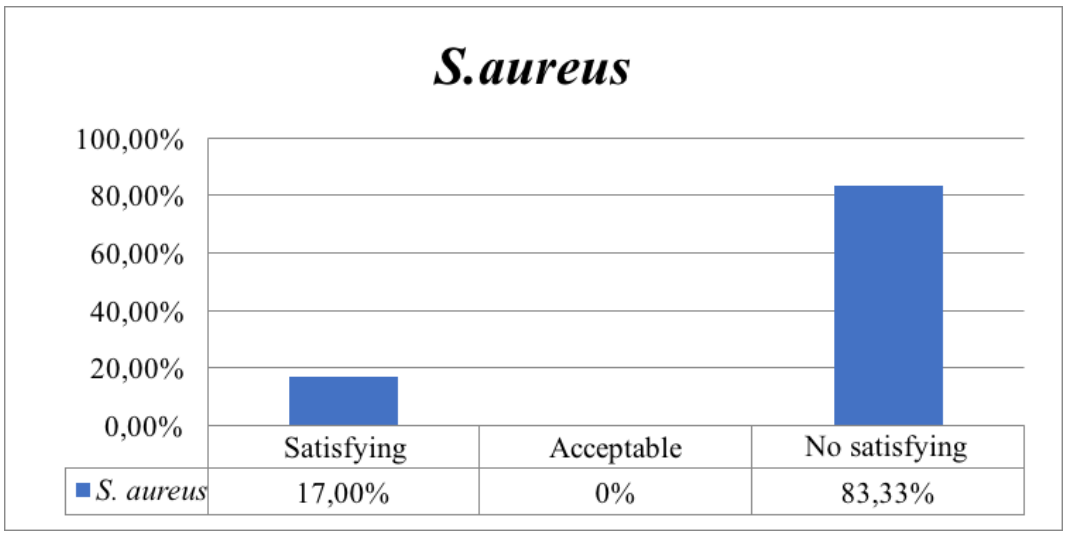

Figure 3. Microbiological quality according to *Staphylococcus aureus*

Referring to the standards of the Luxemburg regulations, 2018, 17.00% of the samples are

satisfactory, no sample is acceptable and 83.33% of the samples are unsatisfactory.

3.3 Microbiological Quality According to Yeasts and Molds

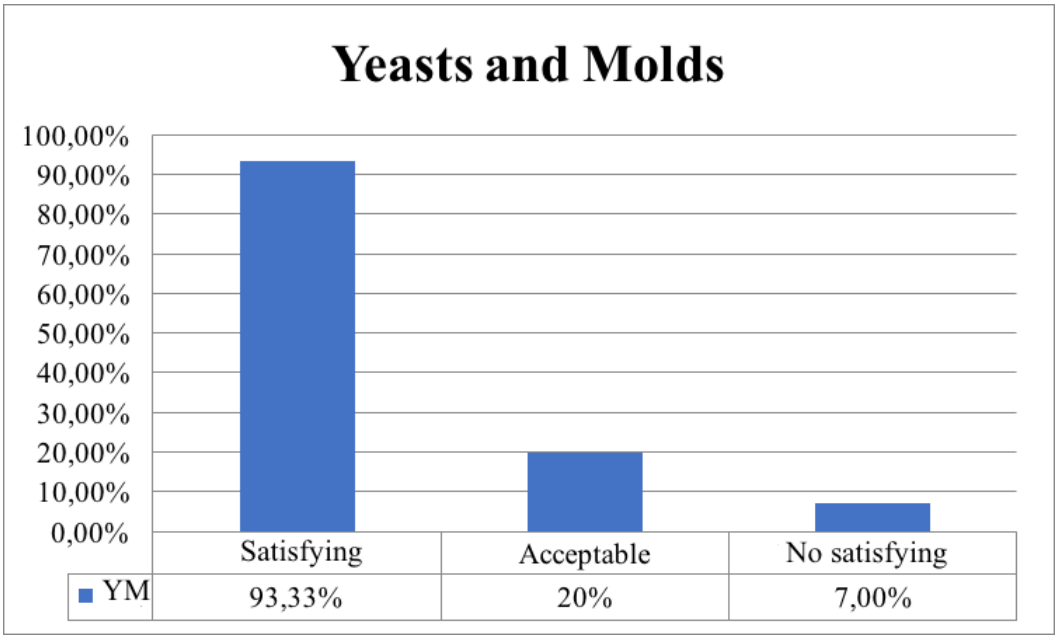

Figure 4. Microbiological quality according to yeasts & molds

According to regulation of the standard set on yeasts and molds at 5x10 4 of Luxemburg, 2018; 93.33% of the samples are satisfactory, 20% of the samples are acceptable and 7.00% of the samples are unsatisfactory.

3.4 Dornic Acidity Results

titratable acidity expressed in degrees Dornic . Table 5 below presents the results of the first ten samples, the results vary between 22 and 29.5 ᴰ and are all higher than the standard (14-18 ᴰ).

Table 5. Dornic acidity values of the first ten samples

Sample	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}
Acidity (ᴰ)	29.5	26	22	25	22.5	24.5	27.5	26.5	23.5	25

S= sample

The results of the Dornic acidity of the second ten samples are presented in Table 6 below. They vary between 22.5 and 28.5 ᴰ and are therefore abnormally high according to the values recommended by the Luxemburg standard (14-18 ᴰ).

Table 6. Dornic acidity values of the second decade of the sample

Sample	E_{11}	E_{12}	E_{13}	E_{14}	E_{15}	E_{16}	E_{17}	E_{18}	E_{19}	E_{20}
Acidity (ᴰ)	25.5	28.5	26.5	23.5	22.5	26	23	23.5	27.5	27

Dornic acidity measurement of the third ten sample is listed in Table 7 below. The results are

between 21.5 and 30.5 °D; this last value is the highest of all the samples and the whole is also above the quality standard.

Table 7. Dornic acidity values of the third decade of the sample

Sample	E_{21}	E_{22}	E_{23}	E_{24}	E_{25}	E_{26}	E_{27}	E_{28}	E_{29}	E_{30}
Acidity (°D)	28	30.5	21.5	29.5	24	26	28	29	28	22.5

4. Discussion

The microbiological parameters taken into account in this study were: TAMF, *E. coli* , staphylococci, yeasts & molds and *Salmonella spp*.

The FMAT count showed dissatisfaction in 20% of cases according to the standard (<$5x10^4$ CFU/ mL) with an average content of $3.1x10^6$ CFU/ mL. Our results are lower than those observed by Kossou et *al* (2007) in Chad who find high loads of FMAT in the rainy and dry season in large-mix milk as well as those of Ma ïwore et *al* (2018) in Cameroon in the ordinary and improved milking conditions where there were approximately 90 and 70% dissatisfaction respectively. Our results are similar to those observed by Aggad (2009) and his collaborators in western Algeria as well as those of Afif et *al* (2008) in Morocco. The highly sought-after FMAT in microbiology characterizes the hygienic quality of food and its contamination; milk is a foodstuff containing very few micro-organisms when it is taken under good hygienic conditions and from a healthy animal; despite its high propensity for contamination.

The search for *E. coli* was positive (norm N<10) in a proportion of 17% with a limiting percentage of 13%. The highest content was that of sample 15 ($1x10^4$ CFU/ mL). This rate of 30% of samples not meeting the standards is similar to that observed by Ma ïwore et *al* in 2018 in Maroua in northern Cameroon. Our results are lower than those observed by Katinan et *al* (2012) in Ivory Coast who found *E. coli* in 51% of curd samples. The presence of this coliform bacillus provides information on faecal contamination and poor hygiene practice by breeders in the sites and at the time of milking. Their lack of education could jeopardize the hygiene of the milk and thus compromise its known safety quality.

The count of staphylococci in our samples was very positive with an average content of $4.6x10^4$ CFU/mL ±0.4 . Figure 3 shows that 83.33% of the samples were unsatisfactory, that is to say had a load greater than the standard 10^2 in Staphylococci. The highest content was that of sample 23, ie $1.7x10^5$ CFU/ mL. Our result is in the same order as those of Ma ïwore and his collaborators as well as Aggad and his collaborators who respectively found a percentage of 70% and 54.28% of staphylococci in their samples. Contrary to our observations, the work of Labioui et *al.* (2009) as well as that of Matallah et *al* (2021) reveal a total absence of staphylococci in raw milk. This high load of staphylococci in our samples could be explained by the state of health of cows which could be affected by mastitis and thus carry the germs on the udder, the hands of the milkers as well as the utensils used during milking. Second, milking was done in the open air and in an unsanitary environment; all these factors contribute to increase the content of these germs in the milk. Staphylococci are bacteria carried by human and animal skin. The hygiene of the milkers and the udder as well

as the unsanitary environment of the milking would therefore be determining factors.

Figure 4 presents the proportion of yeasts & molds observed in our samples. This parameter presents a dissatisfaction of 7% according to the standard. Yeasts & molds are spoilage germs that are usually very rare in milk; rather, they may be useful in the production of milk-derived products by imparting the desired flavor and/or providing deacidification. The loads obtained in our samples lend themselves to this.

With regard to *Salmonella spp* , the results recorded in the three tables show the total absence of *Salmonella spp* in all the samples analyzed. This result is similar to those of Ma ïwore et *al* , Labioui et *al* (2008) as well as those of Matallah et *al* (2021) who did not detect Salmonella in their samples. In the Ivory Coast, however, Katinan et *al* detected 51% of Salmonella in curdled milk, making it unfit for consumption. This absence of Salmonella in our case could be explained by the high acidity of the milk under the action of lactic acid bacteria which, through their activity, inhibit the presence of pathogenic bacteria, possibly *Salmonella spp* . Since salmonella is a very pathogenic bacteria for humans, any food intended for human consumption must be free of it.

titratable acidity values obtained 24 hours after sampling are high with an average of 26.5 °D, oscillating between 22 and 30.5 °D. These results are in the same order as those found by Ma ïwor é et al. (2018) in Maroua in North Cameroon. For the latter, the acidity was between 18 and 26 °D. This rise observed in our case would be linked to the activity of lactic acid bacteria transforming lactose into lactic acid knowing that it is this substrate which is measured. The presence of lactic acid bacteria in milk in Chad has been confirmed by the work of Doutoum and collaborators; these noted the presence of homo fermentatives (Doutoum et *al*., 2013b). In addition, the hygienic conditions, the handling of the milk, the microbial flora and the content of caseins, mineral salts and ions can influence the acidity of the milk. It is therefore known that the acidity of milk increases with time and with the microbial load.

5. Conclusion

This study assessed the physico-chemical and microbiological quality of raw milk. In doing so, the Dornic acidity was very high in all 30 samples with an average of 26.5 °D. The FMAT was unsatisfactory in 20%, E. coli was unsatisfactory in 17%, staphylococci in 83.3% and the yeast & mold parameter with 7% dissatisfaction. *Salmonella spp.* were absent in all samples. These results compromise the safety quality of the milk. Raising awareness of hygiene in the production chain could restore the dissatisfactions observed and thus spare the prized foodstuff from any risk to the health of the consumer.

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