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Physicochemical and Microbiological Quality of *Lait Caillé* (Curdled Milk) Consumed in Korhogo, Côte d'Ivoire

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Abstract

Lait caillé (traditional fermented milk product) is consumed by all age groups of the population. This informally produced food could constitute a real public health problem. The objective of this study is to evaluate the hygienic quality of *Lait caillé* in order to protect the consumer's health. A total of 100 *Lait caillé* samples were taken across the city and analysed. Total aerobic flora, total coliforms, *Escherichia coli*, *Staphylococcus aureus*, yeasts and moulds, pH and titratable acidity were determined. Physicochemical analyses revealed that *Lait caillé* is an acid food (pH = 3.75 ± 0.32). Microbiological analyses showed that the average load of the total aerobic flora is 9.32 ± 8.47 × 10^3 CFU/mL, while that of the total coliforms is 1.15 ± 1.58 × 10^3 CFU/mL. All samples contain less than 1 CFU/mL of *Escherichia coli*, while the average *S. aureus* load is 1.28 ± 1.69 × 10^3 CFU/mL. As for yeasts and moulds, their average load is 1.78 ± 3.34 × 10^3 CFU/mL. These results show that the *Lait caillé* consumed in Korhogo is of poor hygienic quality and constitutes a major public health problem. Antibiotic resistance of *S. aureus* should be analysed in perspective.

Keywords: *Lait caillé*, fermented milk product, hygienic quality, public health

1. Introduction

Milk has been a food consumed by humans since antiquity, because of its richness in nutrients and the benefits it provides to the consumer's health. It contains various nutrients such as carbohydrates, lipids, proteins, vitamins and minerals (Seme, 2015; Arroum, 2016; Learoussy, 2020; Nyokabi, 2021; Dash, 2022). The conservation of this fragile food has always been a major concern for humans. Also, since antiquity, fermentation has been the least expensive method of preserving fresh milk, practiced throughout the world (Mbawala et al., 2014). It results in the consumption of certain constituents of fresh milk by fermentative microorganisms, followed by the production of countless nutrients beneficial to the consumer's health, and above all the lowering of the pH of fermented milk, thus extending its shelf life (Samet-Bali, 2012; Béal, 2019). Thus fermented milk products contain bioactive and probiotic compounds. Fermentation contributes to alleviating lactose intolerance, enriching with vitamins and mineral salts, improving protein digestibility and the organoleptic quality of dairy products (Samet-Bali, 2012; Béal, 2019; Mukisa, 2020).

Yogurt is the most consumed dairy product in the West (Béal and Helinck, 2019), however a wide variety of fermented dairy products are produced around the world. Thus, we have *Kefir* in the Cause, *Koumi*, *Aïran* in Central Asia, *Matsoni* in Georgia, *Aïrag* in Mongolia, *Yakult* in Japan, *Labneh* in Syria (Middle East), *Leben*, *Klila* in the Maghreb, *Dahi*, *Lassi* in India, *Viili* in Finland, *Surmjolk* in Iceland, *Mozzarella* in Italy, *Câilles* in Quebec, *Queso molido* in Mexico, *Ikivuguto* in Rwanda, *Kosam*, *Bongo* in Uganda, *Rayeb* in Tchad, *Pendidam* in Cameroon, *Kaadam*, *Tufam* in Sénégal, *dêguê*, *Lait caillé* in Côte d'Ivoire (Koussou, 2007; Katinan, 2012; Samet-Bali, 2012; Leksir, 2015; Arroum, 2016; Mukisa, 2020; Montes de Oca-Flores, 2021).

Lait caillé, in addition to its tangy taste appreciated by all and its very affordable cost, is an important source of animal origin protein in addition to the diet of the population. Apart from its importance in human nutrition, the dairy product is a favourable medium for the growth of microorganisms such as bacteria, yeasts and moulds which cause its deterioration and adverse effects on the consumer's health (Gunasena, 2021; Dash, 2022). The production of *Lait caillé* in Korhogo remains informal. Thus, this dairy product, produced in a traditional way, could be a source of pathogenic microorganisms' contamination. A poor hygienic quality of the product would therefore constitute a serious public health problem for the population of Korhogo.

This study aims to evaluate the hygienic quality of *Lait caillé* consumed in Korhogo by physicochemical and microbiological analyses in order to preserve the consumer's health.

2. Materials and Methods

2.1. Study Matrix

In Korhogo, *Lait caillé* is produced using two processes: artisanal production and semi-

industrial one. Artisanal production concerns small quantities of product (5 L to 25 L), while semi-industrial production processes at least 50 liters of milk. In addition, artisanal *Lait caillé* (AL) is packaged in recycled plastic bottles or in plastic sachets. While semi-industrial *Lait caillé* (S-IL) is packaged in specific plastic bottles to each dairy, hermetically sealed (often heat sealed) and then labelled. In artisanal production, *Lait caillé* is obtained by fermentation of milk reconstituted from milk powder or fresh cow's milk. Whereas in the case of semi-industrial production, *Lait caillé* is essentially obtained from fresh cow's milk collected from local breeders. In both production methods, the producers use an industrial yogurt from a leading dairy company in Côte d'Ivoire as a ferment.

2.2. Sampling

The samples were taken randomly, in neighborhood shops for semi-industrial *Lait caillé*; in shops, as well as on sites with large population gatherings (markets, bus stations, certain crossroads and around schools) for artisanal *Lait caillé*.

The samples thus obtained are packaged in coolers containing cold condensers and then transported to the laboratory for physicochemical and microbiological analyses. A total of 100 samples [50 artisanal *Lait caillé* (LA) and 50 semi-industrial *Lait caillé* (S-IL)] were collected from February to April 2022 across Korhogo city.

2.3. Physicochemical analyses

The physicochemical parameters analysed in this study are pH and titratable acidity.

The pH was determined using a digital pH meter (Hanna, Portugal) following the manufacturer's instructions. As for the titratable acidity, it was determined by drawing 10 mL of *Lait caillé* with a sodium hydroxide solution (0.1 M/L) in the presence of phenolphthalein according to the AOAC 947.05 method. The result is expressed in Dornic degrees (°D). By definition, 1 °D corresponds to 1 g of lactic acid per 1 L of milk (Vignola, 2002).

2.4. Microbiological analyses

The microbiological analyses were preceded by successive decimal dilutions of each sample of *Lait caillé* according to standard NF V08-010. The successive dilutions (10^{-1} to 10^{-6}) and the stock suspension of each sample were inoculated in duplicate on each selective agar. Total aerobic flora was enumerated on Plate Count Agar, then incubated at 30 °C for 72 hours according to NF/ISO 4833:2003. Total coliforms were enumerated on VRBL agar (Biokar) at 30°C for 24 hours according to NF/ISO Standard method (NF/ISO 4832: 2006). *E. coli* was enumerated on Rapid'*E.coli* 2 (Bio-Rad) at 44 ° C for 24 to 48 hours according to NF/ISO Standard method (NF/ISO 16140: 2013). *Staphylococcus aureus* was enumerated on Baird Parker agar (Bio-Rad) containing tellurite egg yolk emulsion at 37 ° C for 48 hours as describe in NF/ISO 6888: 2004. Fungal flora were enumerated on Sabouraud media with chloramphenicol

(Biokar) at 25 ° C for 5 days according to the NF/ISO 16212: 2011 standard.

3. Results

3.1. Physicochemical characteristics

Out of a total of 100 samples taken at random from the various sales sites in Korhogo city, 50 are of artisanal production and 50 others of semi-industrial production.

The average pH value of all the samples was 3.75 ± 0.32, with 3.8 ± 0.29 and 3.70 ± 0.34 respectively for the artisanal *Lait caillé* (AL) and semi-industrial *Lait caillé* (S-IL) samples. There was no significant difference ($p < 0.05$) between the two types of *Lait caillé* production, as shown in Table 1.

Also, Table 1 indicates that the mean value of the titratable acidity of all the samples was 104.95 ± 22.13 °D, that of (AL) samples was 91.86 ± 14.23 °D, while that of (S-IL) samples was 118.04 ± 20.9 °D. A significant difference ($p < 0.05$) was observed between the two *Lait caillé* production methods.

3.2. Microbiological characteristics

The various microbial loads obtained are presented in Table 2. The microbiological analyses carried out on the 100 *Lait caillé* samples [50 (AL) samples and 50 (S-IL) samples] revealed an average value for the total aerobic flora (TAF) of $9.32 \pm 8.47 \times 10^3$ CFU/mL. Also the mean value for the TAF of the AL and S-IL samples was respectively $9.48 \pm 7.96 \times 10^3$ CFU/mL and $9.15 \pm 9.03 \times 10^3$ CFU/mL. No significant difference ($p < 0.05$) was observed between the two production methods.

The average value for coliforms was $1.15 \pm 1.58 \times 10^3$ CFU/mL. Also, the average coliform value for the AL and S-IL samples was $1.51 \pm 1.88 \times 10^3$ CFU/mL and $7.82 \pm 11.06 \times 10^2$ CFU/mL, respectively. The mean total coliform load of the AL samples was higher ($p < 0.05$) than that of the S-IL samples. These values were higher than that of the French standard (< 10 CFU/mL) (JORF, 1980) for fermented milk products.

The samples analysed contained less than 1 CFU/mL of *E. coli*. These samples comply with the French standard (< 1 CFU/mL) (JORF, 1980). As for *S. aureus*, the average load was $1.28 \pm 1.69 \times 10^3$ CFU/mL. The AL and S-IL samples contain on average $1.99 \pm 2.03 \times 10^3$ CFU/mL and $5.65 \pm 7.47 \times 10^3$ of *S. aureus* respectively. AL samples contained less ($p < 0.05$) *S. aureus* than those of S-IL samples. These average values were higher than the French standard (< 10 CFU/mL) (JORF, 1980).

Table 1: Physicochemical characteristics of *Lait caillé* sold in Korhogo

Lait caillé	**pH**	**Titratable acidity (° D)**
Artisanal *Lait caillé* (AL)	3.8 ± 0.29[a]	91.86 ± 14.23[a]
Semi-Industriel *Lait caillé* (S-IL)	3.7 ± 0.34[a]	118.04 ± 20.9[b]
Overall Mean	3.75 ± 0.32	104.95 ± 22.13

Averages followed by the same lowercase letters (a,b) are not statistically different at the 5 % level

Table 2: Microbiological characteristics of *Lait caillé* sold in Korhogo

Microorganisms	Production method		Overall Mean	Standards
	Artisanal *Lait caillé* (AL)	Semi-Industrial *Lait caillé* (S-IL)		
TAF (10^3 CFU/mL)	9.48 ± 7.96[a]	9.15 ± 9.03[a]	9.32 ± 8.47	> 10^7
Total coliforms (10^3 CFU/mL)	1.51 ± 1.88[a]	0.782 ± 1.106[b]	1.15 ± 1.58	< 10
E. coli (10^3 CFU/mL)	< 1	< 1	< 1	< 1
S. aureus (10^3 CFU/mL)	1.99 ± 2.03[a]	5.65 ± 7.47[b]	1.28 ± 1.69	< 10
Yeasts and Moulds (10^3 CFU/mL)	1.9 ± 2.8[a]	1.66 ± 1.78[a]	1.78 ± 3.34	< 10^2

Averages followed by the same lowercase letters (a,b) are not statistically different at the 5 % level

The average value for yeasts and moulds was 1.78 ± 3.34 × 10^3 CFU/mL with respectively 1.9 ± 2.8 × 10^3 CFU/mL and 1.66 ±1.78 × 10^3 CFU/mL for the AL and S-IL samples. No significant difference ($p < 0.05$) was observed between these two production methods.

4. Discussion

4.1. Physicochemical characteristics

The analyses revealed that the average pH value of all the *Lait caillé* samples was 3.75 ± 0.32, while that of the *Lait caillé* of artisanal production (AL) and that of semi-industrial production (S-IL) was 3.8 ± 0.29 and 3.7 ± 0.34 respectively. From these analyses, it appears that the *Lait caillé* consumed in Korhogo is an acid pH food (pH from 4.5 to 3.7) according to (Naïtali et al., 2017). This acidic pH could constitute an inhibition factor of the growth of neutrophilic microorganisms (pH from 5.5 to 8.0) (Coyette and Mergeay, 2013) among which there are many pathogens (Black, 2012). This mean value is lower than that obtained by (Katinan et al., 2012) (pH from 4.84 ± 0.25 to 5.02 ± 0.16) in *Lait caillé* in Yamoussoukro. Similarly, the results obtained by the work of (Samet-Bali et al., 2012) (pH from 4.45 ± 0.04 to 4.27 ± 0.10) in Tunisia on the *Leben* as well as those of the work of (Zongo et al., 2020) (pH from 3.98 ± 0.03 to 4.39 ± 0.005) on the *Palm Sap-based Kefir Beverage* in Burkina Fasso, are higher than those of this study. However, the results of this study are in agreement with those of (Birali et al., 2019) (pH from 3.5 to 3.9) on *Mashanza* in South Kivu, (Boko et al., 2016) (pH 3.77 ± 0.17) on curdled milk in Benin, as well as those obtained by (Mbawala et al., 2014) (pH from 4.38 ± 0.06 to 3.26 ± 0.05) on the *Pendidam* in Cameroon.

As for the titratable acidity, the average value of all the samples during this study was 104.95 ± 22.13 °D, while that of *Lait caillé* from artisanal production (AL) and that from semi-industrial production (S-IL) was 91.86 ± 14.23 °D and 118.04 ± 20.9 °D respectively. Statistical analyses revealed a significant difference ($p < 0.05$) between the two methods of *Lait caillé* production. There would be more lactic acid in the S-IL samples than in the AL ones. The difference in the level of acidity of the two types of *Lait caillé* would be linked to the fermentation parameters (constant optimum temperature and fermentation time). Indeed, it is very difficult to keep the temperature constant during fermentation due to a lack of appropriate equipment (fermenter). Thus, in the context of (AL), the temperature during fermentation drops very quickly to reach that of the production room (ambient temperature) due to the use of unsuitable fermenters (plastic buckets, kitchen utensils). As for (S-IL), the fermentation of large quantities of milk would require the use of adequate equipment. The optimum temperature / fermentation time couple would influence the acidity of the *Lait caillé*. The more constant the optimum temperature and the longer the fermentation time, the higher the titratable acidity and the lower the pH. The tangy flavor of *Lait caillé*, appreciated by consumers, would be linked to compliance with the optimum temperature / fermentation time couple parameter during the fermentation of the milk.

The average titratable acidity value of (AL) is close to that obtained during the study of (Mbawala et al., 2014) (93.31 ± 2.19 °D) on *Pendidam* in Cameroon, but higher than that resulting from the work of (Samet-Bali et al., 2012) (65.55 ± 0.10 °D to 71.35 ± 0.54 °D) on *Leben* in Tunisia and lower than the results of (Katinan et al., 2012) on *Lait caillé* in Côte d'Ivoire and (Boko et al., 2016) on curdled milk in Benin, with respectively (101.77 ± 16.8 °D – 113.53 ± 17.48 °D) and 156.36 ± 30.22 °D. As for (S-IL), the average value of titratable acidity obtained during this study is higher than the results of the work of (Birali et al., 2019) (41.5 °D – 100.8 °D) on *Mashanza* in South Kiwu and (Samet-Bali et al., 2012) (65.55 ± 0.10 °D to 71.35 ± 0.54) on *Leben* in Tunisia, but lower than the results of (Boko et al., 2016) (156.36 ± 30.22 °D) on curdled milk in Benin. This acidity of the samples could have an inhibiting effect on the growth of neutrophil bacteria in *Lait caillé*.

4.2. Microbiological characteristics

The average value of the Total Aerobic Flora (TAF) for all the samples during this study was $9.32 \pm 8.47 \times 10^3$ CFU/mL, likewise that of (AL)

and (S-IL) was respectively 9.48 ± 7.96 × 10^3 CFU/mL and 9.15 ± 9.03 × 10^3 CFU/mL. No significant difference ($p < 0.05$) was observed between the two production methods. This average value does not comply with the French 10^3 CFU/mL (JORF, 1980) and Luxembourg (10^7 CFU/mL, F-054 Rev05) standards. Also, this average value is lower than that obtained by the study of (Katinan et al., 2012) (8.20 ± 9.1×10^4 CFU/mL to 1.47 ± 0.98×10^5 CFU/mL) on *Lait caillé* in Côte d'Ivoire, but higher than that obtained by (Birali et al., 2019) (9.8 × 10^2 CFU/mL) on *Mashanza* in South Kivu.

In addition, 21/100 or 21% of the *Lait caillé* samples analysed have a load of less than 1 CFU/mL (< 1 CFU/mL) in FAT. However, *Lait caillé* is a fermented milk product, obtained by fermentation of milk by lactic fermentative microorganisms. In general, in Côte d'Ivoire, *Lait caillé* manufacturers use industrial ferments (yoghurt pot). A single brand of yoghurt (from a leading dairy company in Côte d'Ivoire) is generally used as a ferment. If 21% of the *Lait caillé* samples analysed contain less than 1 CFU/mL (< 1 CFU/mL) in FAT, this suggests that this ferment does not contain microorganisms. It could be that yogurt is obtained by enzymatic fermentation as is increasingly common (Budak and Akal, 2018). However, according to the *Codex Alimentarius*, yogurt is defined as being a "coagulated milk product obtained by lactic fermentation thanks to the action of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* from milk (pasteurized, concentrated, partially skimmed enriched in dry extract)" (FAO/WHO, 1975). Also, European Directive 79/112/EEC of December 18, 1978 and *Codex Stan* 243-2003 stipulate that the name "yogurt" can be refused if "the presence of live lactic acid bacteria in abundant quantity" is not verified. From these analyses, it appears that the pot of industrial ferment used in the production of *Lait caillé* should not have the name of "yogurt" but that of "fermented milk". Consequently, this "yogurt" would not be recommended for people who consume it with a view to reseeding their digestive tract with lactic acid bacteria (*Lactobacillus delbrueckii* susp. *bulgaricus* and *Streptococcus thermophilus*) following prolonged medical treatment with antibiotics.

The average value of the total coliform load of all the *Lait caillé* samples in this study was 1.15 ± 1.58 × 10^3 CFU/mL. Also, the average coliform value for the AL and S-IL samples was 1.51 ± 1.88 × 10^3 CFU/mL and 7.82 ± 11.06 × 10^2 CFU/mL, respectively. A significant difference ($p < 0.05$) was observed between these two types of *Lait caillé*. The presence of total coliforms indicates that this food would be produced in non-compliance with good hygiene practices. These samples are therefore unfit for human consumption in view of international standards. Given the acid pH of these samples, the presence of these microorganisms could be explained by an adaptation of certain bacteria to a progressive acidification of their environment. These coliforms could come from water, equipment, personnel and the production environment.

Moreover, this average value is close to that obtained by the work of (Samet-Bali et al., 2012) in Tunisis on the *Leben*, but lower than that obtained by (Katinan et al., 2012) (from 2.80 ± 4.86×10^4 CFU/mL to 7.12 ± 6.32×10^4 CFU/mL) on *Lait caillé* in Côte d'Ivoire and higher than that obtained by (Koussou et al., 2007) (226.75 ± 84.78 CFU/mL) in Tchad on the *Rayeb*.

The presence of less than 1 CFU/mL (< 1 CFU/mL) of *E. coli* in all the samples analysed could be explained by an improvement in compliance with hygiene rules during the production of *Lait caillé*. Indeed, the agents of Health Ministry (Public Hygienic Institute) regularly carry out awareness campaigns on hygiene, and carry out inspection visits to the establishment where water (bottle or sachet), refreshing drinks and dairy products are produced in Korhogo city and its region. It is an agro-pastoral region where the consumption of milk and dairy products is a dietary habit of the populations. However, some authors have recorded the presence of *E. coli* in artisanal dairy products. Thus (Katian et al., 2012) (from 3.80 ± 7.2×10^3 CFU/mL to 2.41 ± 4.16×10^4 CFU/mL), (Millogo et al., 2018) (13.43 ± 35.29 CFU/mL) in

Burkina Faso and (Birali et al., 2019) (from 0 to 1.6×10^2 CFU/mL) in South Kiwu recorded high levels of *E. coli* during their various studies.

The mean value of the *S. aureus* load of all the samples was $1.28 \pm 1.69 \times 10^3$ CFU/mL, while that of the AL and S-IL samples was respectively $1.99 \pm 2.03 \times 10^3$ CFU/mL and $5.65 \pm 7.47 \times 10^3$ CFU/mL. This value does not comply with the Luxembourg standard for dairy products (< 10 CFU/mL, F-054 Rev05). These products could pose a health risk to the consumer. Indeed, *S. aureus* produces exotoxins, responsible for collective toxi-infections (Assous, 1999; Delarras, 2014). The presence of *S. aureus* in *Lait caillé* would be linked to the lack of personal hygiene of producers. Indeed, humans are an important reservoir of this microorganism (Delarras, 2014; Naïtali, 2017). This average value is not in agreement with the results of the study of (Birali et al., 2019) and (Zogo et al., 2020), who found less than 1 CFU/mL in the analysed samples. It is also higher than that obtained by (Millogo et al., 2018) (15 ± 17.8 CFU/mL) in local yoghurt in Burkina Faso.

As for the average value of the yeast and mould load of all the samples, it was $1.78 \pm 3.34 \times 10^3$ CFU/mL, while that of the AL and S-IL samples was respectively $1.9 \pm 2.8 \times 10^3$ CFU /mL and $1.66 \pm 1.78 \times 10^3$ CFU/mL. This average value is close to the results of the study of (Birali et al. 2019) (1.5×10^3 CFU/mL) on *Mashanza* in South Kivu. The growth of yeasts and moulds would be favored by the acid pH of *Lait caillé*. Indeed, these microorganisms are acidophilic (Pitt, 2009, Kumar, 2016). They would come from the contamination of raw materials (milk powder or fresh milk), water, utensils and the production environment. Their presence in *Lait caillé* would be linked to the germination of spores (for sporulated species) or to post-pasteurization contamination. They would constitute a danger for the consumer, if however they contained strains capable of producing secondary metabolites (mycotoxins) which could have an adverse effect on the consumer's health.

5. Conclusion

This study, whose objective was to evaluate the hygienic quality of *Lait caillé* consumed in Korhogo, has shown, in view of the various results that this food is of poor hygienic quality. Indeed, the total coliform load above standards indicates a lack of hygiene during the various stages of *Lait caillé* production. This study also revealed the presence of *S. aureus*, yeasts and moulds in proportions exceeding the international standards for these microorganisms. Thus, artisanal *Lait caillé* (AL) is higher in total coliforms, yeasts and moulds, while semi-industrial *Lait caillé* (S-IL) is higher in *S. aureus*. Also, this study revealed the absence of *E. coli* in the samples indicating the absence of faecal contamination of *Lait caillé*. The health checks carried out by the agents of Public Hygienic Institute have contributed to significantly improving the hygienic quality of this food. However, a census campaign and training in good hygiene and production practices for all producers would help to improve the sanitary quality of *Lait caillé*, thus protecting consumer's health. Also, a study of isolated strains of *S. aureus* in relation to their possible resistance to antibiotics should be carried out in perspective, with a view to evaluating the extent of this phenomenon in Korhogo.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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