

# Biochemical and Microbiological Characteristics of Raw Milk and Curdled Milk Originated from the Central Region of Burkina Faso

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**Abstract** Milk and dairy products play an important role in human nutrition in Burkina Faso as in several developing countries. This study aimed to describe the curdled milk process in the Central region of Burkina Faso through a monitoring of the production and to study the biochemical and microbiological characteristics of raw milk and curdled milk. Microbiological and biochemical characteristics of the products were determined using standard methods. Biodiversity of lactic acid bacteria in curdled milk was determined using (GTG) 5-PCR and 16S rRNA gene sequencing. Processing of curdled milk takes place in calabashes or plastic containers, without heat treatment of raw milk. The fermentation is spontaneous and lasts for 24 - 48 h at ambient temperature. Biochemical analysis showed a low mean pH of curdled milk ( $4.34 \pm 0.10$ ) compared to that of raw milk ( $6.47 \pm 0.02$ ). Dry matter (DM), protein and lipid contents were respectively 7.85%; 16.96 g/L DM and 37.11 g/L DM for raw milk while those of curdled milk were 15.85%; 20.85 g/L DM and 27.03 g/L DM. An increase from 0.22 to 0.37 g/L DM of phosphorus and from 1.10 to 2.46 of calcium was observed with the fermentation. However, iron and zinc contents obtained were 0.55 mg/L DM and 1.97 mg/L DM for raw milk and 0.27 mg/L and 1.6 mg/L for curdled milk, respectively. Microbiological analyzes indicated a high number of aerobic mesophilic bacteria for raw milk ( $1.9 \cdot 10^7$  UFC/mL) as for curdled milk ( $3.9 \cdot 10^8$  UFC/mL). In addition, Enterobacteriaceae counts from raw milk and curdled milk were higher than the acceptable limit of  $10^2$  CFU/mL for dairy products. *Lactobacillus fermentum*, *Enterococcus durans/feacium*, *Lactococcus lactis*, *Lactobacillus helveticus* and *Enterococcus feacalis* were the predominant species identified in the curdled milk.

**Keywords:** raw milk, curdled milk, characterization, Lactic acid bacteria, Burkina Faso

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## 1. Introduction

Breeding plays an important role in Burkina Faso's economy and also contributes to the food and nutrition security of populations by providing them, high nutritional value products such as milk. The potential for milk production is estimated around 250 million liters per year and has been steadily increasing over the last twenty years [1]. The milk produced is sold to dairy processing units or directly to consumers by breeders and small traders. Apart from that, it is transformed traditionally to curdled milk

known as *lait caillé* by typically the Fulani people [2]. This curdled milk is prepared from a spontaneous fermentation of raw cow milk or more seldom from raw goat milk. It is consumed as a beverage or in combination with various cereal based products. Formerly produced and consumed in the family, curdled milk is increasingly popular and marketed in large cities and is an important source of income for stakeholders, hence its socioeconomic importance. In addition, milk and dairy products as spontaneous fermented milk contain several 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 [3].

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 [4]. Indeed, previous studies on the quality of curdled milk in Burkina Faso have revealed a poor hygienic quality with high level of enterobacteria, enterococci and other presumptive pathogens [2,4]. This poor quality of milk and milk products was attributed by these authors to the unhygienic conditions of milking, transport, packaging, processing and marketing.

The microbial biodiversity of the traditional fermented milk of the Fulani ethnic group from the North region of Burkina Faso has been described by [5] who showed that the predominant lactic acid bacteria identified in this product were *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Streptococcus*, and *Enterococcus*. In a recent investigation, indigenous LAB and yeasts involved in the fermentation of *lait caillé* samples from bovine milk in the South West area of Burkina Faso were identified [2]. This study revealed predominance of *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Weissella paramesenteroides*, *Lactococcus lactis*, *Enterococcus* spp., *Candida parapsilosis* and *Saccharomyces cerevisiae* [2]. However, few published data exist on the characteristics of the curdled milk produced in the Central region of Burkina Faso where its demand is increasing. The objective of this study is therefore to describe the process of curdled milk produced in the Central region of Burkina Faso and to determine the biochemical and microbiological characteristics of raw milk and curdled milk taken from house-hold production sites.

## 2. Material and Methods

### 2.1. Survey on Curdled Milk Production and Sampling

A survey on the processing of curdled milk was conducted in two localities of the Central region of Burkina Faso, precisely in the communes of Ouagadougou and Loumbila. In the commune of Ouagadougou, tree districts were visited namely Hamdalaye (2 sites), Yagma (1 site) and 14 yaar (1 site). In the commune of Loumbila, one site was visited. After discussion and following of the technology with the producers, a general process for curdled milk production was proposed. A total of 20 milk samples including 10 samples of raw milk and 10 samples of curdled milk were aseptically collected in two separate occasions (5 samples of raw milk and 5 samples of curdled milk at each occasion) using a sterile glass jars at the processing sites. Each sample consisting of about 500 mL of raw milk or curdled milk was placed in a thermo-cooler containing ice blocks, transported to the laboratories of Département Technologie Alimentaire (DTA/IRSAT/CNRST) and stored at 4°C for analyses within 24 h.

### 2.2. Biochemical Analyses

The pH of the samples was measured with an electronic pH-meter (Consort P901, Belgium) using 100 mL of milk

sample. Dornic acidity of the milk was determined by titration using NaOH (N/9) according to AOAC (2005). The moisture and dry matter contents were determined by drying the sample at 105 °C ± 2 °C for 12 h according to the standard ISO 712 (2009). Ash content was determined by incineration at 550 °C for 4 h according to the standard ISO 2171 (2007). Proteins content was determined by the Kjeldahl method after acid digestion according to the standard AFNOR NF V03 50 (1970). Fat content was determined with Soxhlet apparatus using n-hexane according to the standard ISO 659, (1998). The determination of mineral elements (iron, phosphorus, calcium and zinc) was carried out by flame atomic absorption spectrometry (Perkin-Elmer model 303) according to AOAC (2005).

### 2.3. Microbiological Analyses

Ten grams of each sample were aseptically homogenized with 90 mL of sterile diluent (0.1% peptone, 0.8% NaCl, pH 7.0 ± 0.2) in a stomacher bag (stomacher 400 lab blender, England) at normal speed for 2 min to obtain 10<sup>-1</sup> dilution. Serial dilutions were made from the homogenate of all samples using 9 mL sterile diluent. From appropriate ten-fold dilutions, Aerobic Mesophilic Bacteria (AMB) were enumerated by pour plate on Plate Count Agar (Liofilchem, Spain) incubated aerobically at 30 °C for 72 h according to ISO (International Standard organization) 4833 (2003). Lactic Acid Bacteria (LAB) were enumerated on Man, Rogosa and Sharpe (MRS) agar (Liofilchem, Spain), incubated anaerobically in an anaerobic jar with anaerocult (Merck) at 37 °C, for 72-96 h according to ISO 15214 (1998). After incubation, plates of PCA and MRS containing 15-300 colony forming units (CFU) were counted and results expressed as CFU/mL of sample. Yeasts and molds were enumerated by pour plate on Sabouraud agar (Liofilchem, Spain) incubated at 25 °C for 3-5 days according to ISO 7954 (1988). Enterobacteria were enumerated on Violet Red Bile Glucose (VRBG) agar (Liofilchem, Spain), incubated at 37 °C for 24 h according to ISO 7402 (1993). After incubation, plates of Sabouraud and VRBG containing 15-150 colony forming units (CFU) were counted and results expressed as CFU/mL of sample. Microbial enumerations were conducted in duplicate and means and standard deviation were calculated.

#### 2.3.1. Isolation and Preliminary Phenotypic Characterization of LAB Isolates from Curdled Milk

For isolation of lactic acid bacteria, 15-20 colonies from the highest dilution or suitable plate of MRS were picked and purified by successive streaking on MRS agar under anaerobic conditions as described by [6]. Pure cultures were maintained at -80°C in MRS broth (Liofilchem, Spain) containing 20% (v/v) glycerol. Working cultures were kept at 4°C on MRS agar. The isolates were first characterized based on colony and cell morphology using phase contrast microscope (Olympus optical, BX 40F-3, JAPAN). Gram reaction was carried out by the KOH (3%) method [7]. Catalase production was determined using H<sub>2</sub>O<sub>2</sub> solution (30%). Oxidase reaction was carried out using oxidase disc.

### 2.3.2. Molecular Characterization of LAB Isolates

The isolates of presumptive LAB were streaked on MRS agar and incubated anaerobically at 37°C for 48 h. DNA from pure colonies was extracted using the InstaGene Matrix extraction kit (Bio-Rad Laboratories, Hercules, CA USA) according to the manufacturer's instructions. The extracted DNA was then stored at -20°C for later use. The repetitive extragenic palindromic-Polymerase Chain Reaction (Rep-PCR) amplification method described by [8] was used for the grouping of isolates. The reaction mixture (25 µL) consisted of 13 µL of 2x PCR Master Mix/Dream Taq Green, 4 µL of sterile pure water, 5 µL of primer (5'- GTGGTGGTGGTGGTG -3') (5 µM) and 3 µL DNA (50 ng.µL<sup>-1</sup>) of DNA. The amplification program (SureCycler 8800 thermal cycler, Agilent Technologies, USA) was as follow: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing 45°C for 1 min, extension 65°C for 8 min; final elongation step at 65°C for 16 min, holding at 4°C. PCR products were separated by 1.5% agarose gel electrophoresis in 1.5 × TBE (5 h, 140 V) using a Generuler 1 kb DNA ladder as reference (Fermentas, Vilnius, Lithuania). DNA fragments were stained with ethidium bromide solution (4 µg/L) and photographed (Alpha imager system, Alpha Innotech, USA). Cluster analysis of the rep-PCR profiles were performed using the BioNumerics 4.5 software (Applied Maths, Sint-Martens-Latem, Belgium), as described by [8].

Based on the rep-PCR clusters, representative isolates were selected for 16S rRNA gene sequencing using the universal primers 27f (5'- AGAGTTTGATCMTGGCTCAG - 3') and 1510r (5'- AAGGAGGTGATCCAACCGCA - 3'), as previously described by [9]. Sequencing was performed

by a commercial facility (Macrogen, Inc. Korea). 16S rRNA sequences were manually corrected and aligned using Chromas 2.33 (Technelysium) and CLC Genomics (CLC Bio, Aarhus, Denmark). Subsequently, the corrected nucleotide sequences were aligned to the 16S rRNA gene sequences in the GenBank database using the BLAST algorithm [10] and in EzTaxon database as described by [11].

### 2.4. Statistical analyses of the data

The curves, the averages and the standard deviation were obtained using Microsoft Excel 2013.

## 3. Results

### 3.1. Curdled Milk Production Technology

The curdled milk is obtained by spontaneous fermentation of non-pasteurized fresh cow milk. Figure 1 showed the main steps for the curdled milk production. The fresh cow milk collected in a plastic bucket (Figure 1a) was first filtered (pore size of approximately 1 mm, Figure 1b). Then, transferred to the fermentation container (generally calabash or plastic container covered with a straw-woven lid), and left for fermentation at ambient temperature (34-38 °C) in the house for 24 to 48 h (Figure 1c). The fermentation is stopped when the producer judges that the optimal characteristics of the fermented product are reached. The obtained curdled milk (Figure 1d) can be consumed as a beverage or in combination with various cereal based products.

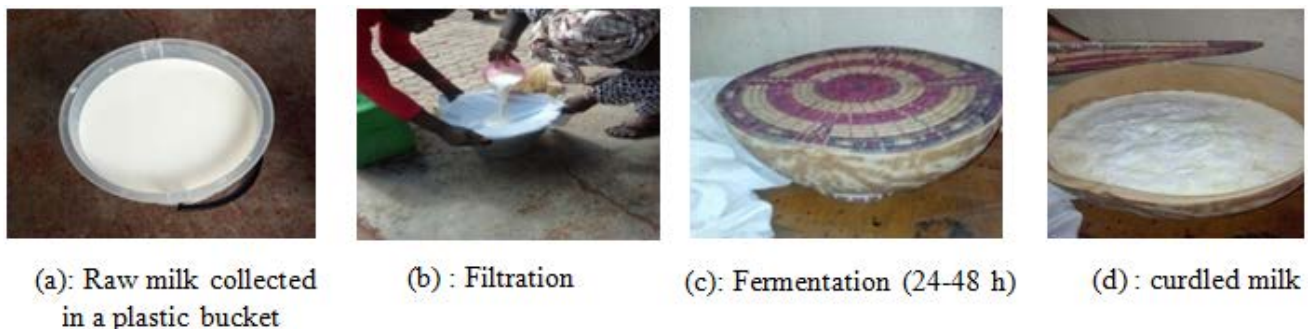


Figure 1. Pictures of the different steps of curdled milk production

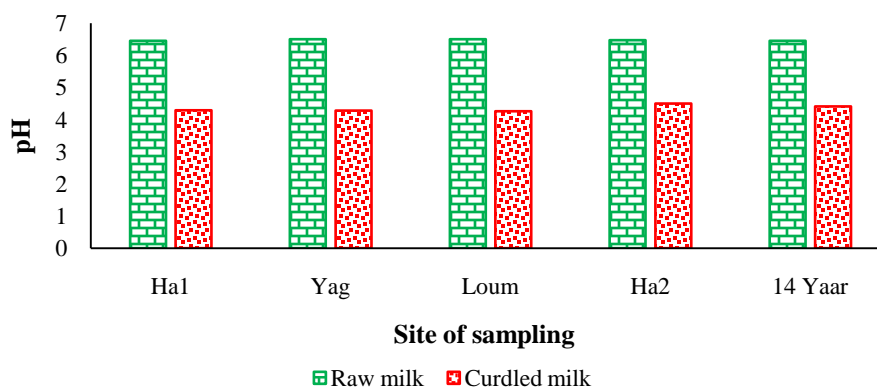
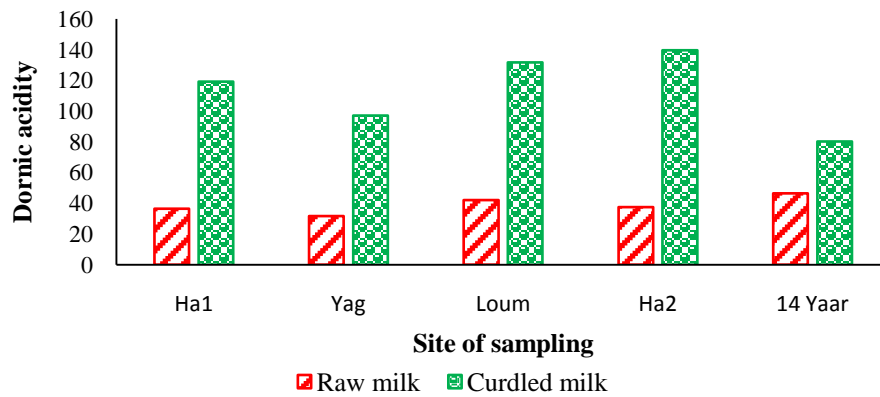


Figure 2. pH of raw milk and curdled milk (Legend: Ha1: Hamdalaye site 1; Yag : site Yagma ; Loum : site Loumbila ; Ha2 : Hamdalaye site 2 ; 14 Yaar: site Secteur 14 yaar)



**Figure 3.** Acidity of raw milk and curdled milk from production site (Legend: Ha1: Hamdalaye site 1; Yag : site Yagma ; Loum : site Loumbila ; Ha2 : Hamdalaye site 2 ; 14 Yaar: site Secteur 14 jaar)

### 3.2. Biochemical Characteristics of Raw Milk and Curdled Milk

The pH of the raw milk ranged from 6.45 to 6.50 with an average of  $6.47 \pm 0.02$  for all the samples analyzed. For the curdled milk, the pH varied from 4.26 to 4.50 with an average of  $4.34 \pm 0.10$  (Figure 2).

Figure 3 show the acidity of raw milk and curdled milk. The acidity varied from  $31 \text{ }^\circ\text{D}$  to  $46.5 \text{ }^\circ\text{D}$  with an average of  $38.82 \text{ }^\circ\text{D} \pm 5.03$  for raw milk while it ranged from  $80.30 \text{ }^\circ\text{D}$  to  $139.62 \text{ }^\circ\text{D}$  with an average of  $113.58 \text{ }^\circ\text{D} \pm 24.61$  for curdled milk.

The biochemical composition of raw and curdled milk is presented in Table 1. The moisture content ranged from 88.8% to 94% for raw milk samples and from 80.26% to 87.05% for curdled milk samples with averages of  $91.73\% \pm 2.7$  and  $84.14 \pm 3.5$ , respectively. The dry matter content ranged from 5.48% to 10.69% for raw milk samples with an average of  $7.85\% \pm 2.5$  while that of curdled milk ranged from 12.94% to 19.73% with an average of  $15.85 \pm 3.5\%$ . Protein levels in raw milk ranged from 15.75 to 18.22 g/L with an average of  $16.96 \pm 1.23$  g/L. There is an increase in the protein content with fermentation. In fact, the curdled milk showed protein content ranging from

18.09 to 24.41 g/L with an average of  $20.85 \pm 3.23$  g/L while the raw milk samples showed an average crude fat content of  $37.11 \pm 9.57$  g/L. A decrease in the lipid content with fermentation was observed with mean values ranging from  $37.11 \pm 9.57$  for raw milk to  $27.03 \pm 5.95$  g/L for curdled milk.

The raw milk samples had an average phosphorus content of  $0.22 \pm 0.07$  g/L. It was observed an increase in phosphorus content in the curdled milk with an average of  $0.37 \pm 0.01$  g/L (Table 1). The iron content of raw milk samples ranged from  $0.26 \pm 0.09$  to  $0.72 \pm 0.03$  mg/L with an average of  $0.55 \pm 0.25$  mg/L. After fermentation, the iron content decreased from  $0.55 \pm 0.25$  to  $0.27 \pm 0.06$  mg/L in the curdled milk. Analysis of raw milk samples presented an average calcium value of  $1.10 \pm 0.66$  g/L. An increase in the calcium content was observed after the fermentation with an average content of  $2.46 \pm 0.33$  g/L for curdled milk. A decrease in zinc content was observed during the spontaneous fermentation of raw milk into curdled milk (Table 1). The raw milk samples had an average zinc content of  $1.97 \pm 0.3$  mg/L with a minimum value of  $1.7 \pm 0.9$  mg/L and a maximum value of  $2.3 \pm 0.5$  mg/L. The zinc content of the curdled milk samples ranged from  $1.1 \pm 0.064$  to  $1.9 \pm 0.2$  mg/L with an average of  $1.6 \pm 0.44$  mg/L.

**Table 1. Biochemical characteristics of raw milk and curdled milk**

Samples	Moisture (%)	Dry matter (%)	Proteins (g/L DM)	Fat (g/L DM)	P (g/L DM)	Fe (mg/L DM)	Ca (g/L DM)	Zn (mg/L DM)
<b>Raw milk (n= 10)</b>	$91.73 \pm 2.7$	$7.85 \pm 2.5$	$16.96 \pm 1.23$	$37.11 \pm 9.57$	$0.22 \pm 0.07$	$0.55 \pm 0.25$	$1.10 \pm 0.66$	$1.97 \pm 0.3$
<b>Curdled milk (n=10)</b>	$84.14 \pm 3.5$	$15.85 \pm 3.5$	$20.85 \pm 3.23$	$27.03 \pm 5.95$	$0.37 \pm 0.01$	$0.27 \pm 0.06$	$2.46 \pm 0.33$	$1.6 \pm 0.44$

**Table 2. Microbiological characteristics of raw milk and curdled milk**

Microorganism counts (CFU/mL)						
Samples	Site of sampling	Aerobic mesophilic bacteria	Enterobacteria	Lactic acid bacteria	Yeasts and molds	
Raw milk	Hamdalaye 1	$2.4 \cdot 10^7$	$3.1 \cdot 10^3$	$1.5 \cdot 10^5$	$7.2 \cdot 10^4$	
	Hamdalaye 2	$4.7 \cdot 10^6$	$1.9 \cdot 10^2$	$3.3 \cdot 10^6$	$7.4 \cdot 10^4$	
	Yagma	$2.8 \cdot 10^7$	$1.4 \cdot 10^3$	$2.1 \cdot 10^5$	$1.4 \cdot 10^4$	
	Loumbila	$3.9 \cdot 10^5$	$1 \cdot 10^4$	$4.8 \cdot 10^4$	$2.7 \cdot 10^3$	
	14 jaar	$4.2 \cdot 10^7$	$2.5 \cdot 10^3$	$9.5 \cdot 10^5$	$1 \cdot 10^2$	
<b>Mean</b>		<b><math>(1.9 \pm 1.7) \cdot 10^7</math></b>	<b><math>(3.4 \pm 3.8) \cdot 10^3</math></b>	<b><math>(3.3 \pm 3.5) \cdot 10^5</math></b>	<b><math>(3.2 \pm 3.2) \cdot 10^4</math></b>	
Curdled milk	Hamdalaye 1	$4.7 \cdot 10^8$	$1.9 \cdot 10^5$	$3.3 \cdot 10^8$	$5.0 \cdot 10^5$	
	Hamdalaye 2	$5.0 \cdot 10^8$	$1 \cdot 10^5$	$2.9 \cdot 10^8$	$3 \cdot 10^6$	
	Yagma	$2.8 \cdot 10^8$	$1.4 \cdot 10^5$	$2.1 \cdot 10^8$	$1.4 \cdot 10^5$	
	Loumbila	$5.8 \cdot 10^8$	$1.0 \cdot 10^2$	$9.9 \cdot 10^8$	$1.6 \cdot 10^7$	
	14 jaar	$1.5 \cdot 10^8$	$7 \cdot 10^5$	$2 \cdot 10^8$	$3 \cdot 10^3$	
<b>Mean</b>		<b><math>(3.9 \pm 1.7) \cdot 10^8</math></b>	<b><math>(2.2 \pm 2.7) \cdot 10^5</math></b>	<b><math>(4.0 \pm 3.3) \cdot 10^8</math></b>	<b><math>(3.9 \pm 6.8) \cdot 10^6</math></b>	



### 3.3. Microbial Counts of Raw Milk and Curdled Milk

The microbial counts of raw milk and curdled milk are presented in Table 2. The AMB counts ranged from  $3.9 \cdot 10^5$  to  $4.2 \cdot 10^7$  CFU/mL with an average of  $1.9 \pm 1.7 \cdot 10^7$  CFU/mL for raw milk samples. For curdled milk, the AMB counts ranged from  $1.5 \cdot 10^8$  to  $5.8 \cdot 10^8$  CFU/mL with an average of  $3,9 \pm 1.7 \cdot 10^8$  CFU/mL. For LAB, the average count was  $3.3 \pm 3.5 \cdot 10^5$  CFU/mL for raw milk and  $4.0 \pm 3.3 \cdot 10^8$  CFU/mL for curdled milk. The variation observed for

LAB counts for the different samples was  $4.8 \cdot 10^4$  to  $3.3 \cdot 10^6$  CFU/mL and  $2 \cdot 10^8$  to  $9.9 \cdot 10^8$  CFU/mL for raw milk and curdled milk, respectively. Enterobacteria count ranged from  $1.9 \cdot 10^2$  to  $1 \cdot 10^4$  CFU/mL for raw milk and from  $1.0 \cdot 10^2$  to  $7 \cdot 10^5$  CFU/mL for curdled milk. The average of enterobacteria count was  $3.4 \pm 3.8 \cdot 10^3$  CFU/mL for raw milk and  $2.2 \pm 2.7 \cdot 10^5$  CFU/mL for curdled milk. The load of yeasts and molds varied from  $1 \cdot 10^2$  to  $7.4 \cdot 10^4$  with an average of  $3.2 \pm 3.2 \cdot 10^4$  CFU/mL for raw milk samples while that of curdled milk samples varied from  $3 \cdot 10^3$  to  $1.6 \cdot 10^7$  CFU/mL with a mean value of  $3.9 \pm 6.8 \cdot 10^6$  CFU/mL.

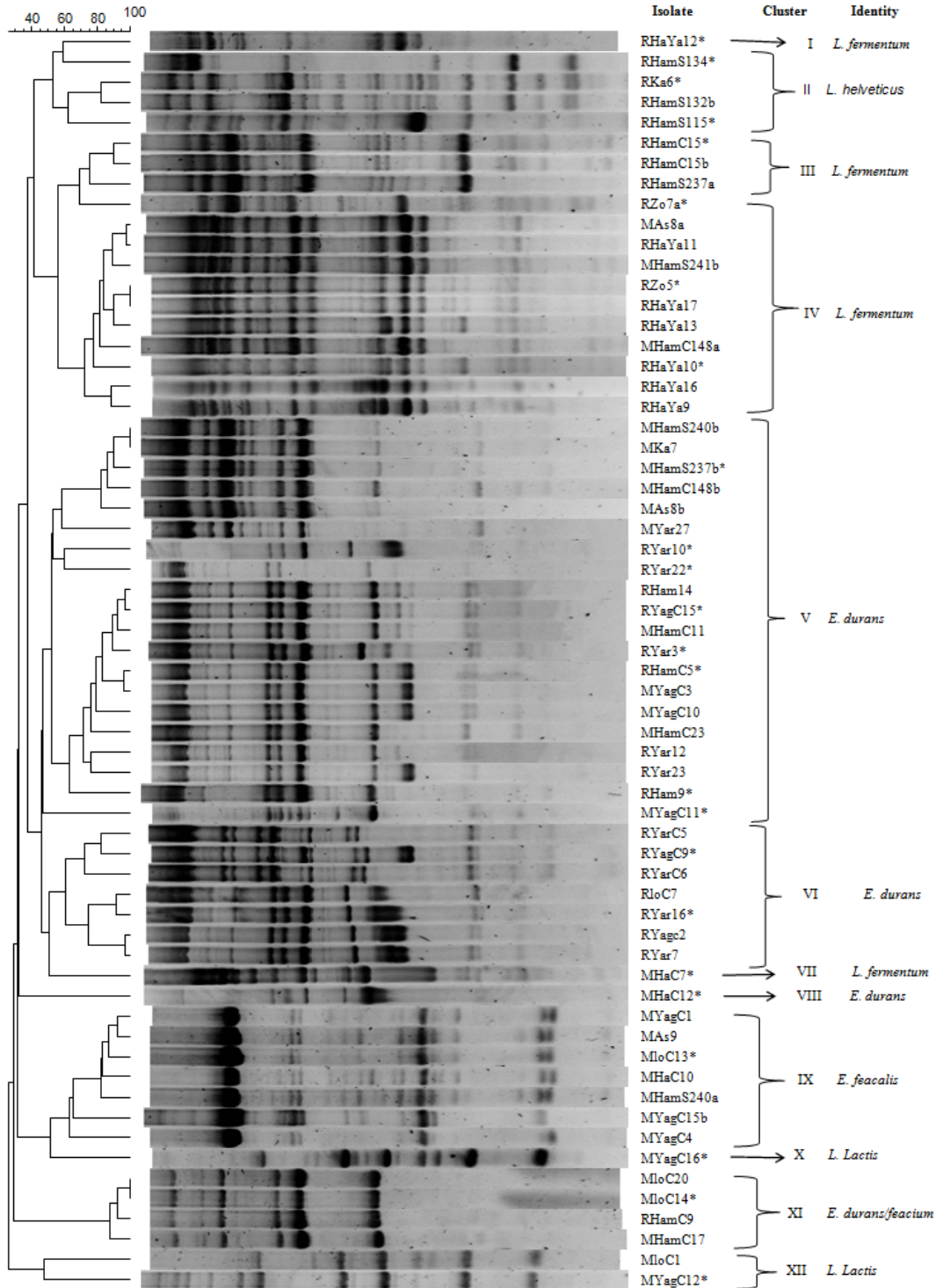


Figure 4. Dendrogram of Rep-PCR DNA profiles (GTG5) of LAB isolated from Ouagadougou and Loubila curdled milk. The dendrogram is based on the Dice similarity coefficient and the unweighted pairing method with arithmetic averages (UPGMA). \* Strains whose 16S rRNA has been sequenced.

### 3.4. Identification of LAB Isolated from the Curdled Milk

A total of 62 non-motile, Gram positive, catalase negative, oxidase negative, rod or coccoid isolates obtained from the curdled milk were presumptively identified as LAB. The isolates were clustered by (GTG) 5-based rep-PCR, dividing them into 12 clusters. Representative isolates of each cluster were identified based on their 16S rRNA gene sequence followed by BLAST search at EzBioCloud (Figure 4). The isolates of clusters I, III, IV and VII (25.8% of the total of LAB isolates) were identified as *Lactobacillus fermentum* with 98.7-99.74% similarity to EzBioCloud sequences. The isolates of cluster II (6.45% of the total LAB isolates) were identified as *Lactobacillus helveticus* with 99.6% similarity to EzBioCloud sequences. The isolates of clusters V, VI and VIII, representing 45.16% of the total LAB isolates were identified as *Enterococcus durans* with 98.94-100% similarity to EzBioCloud sequences. The isolates of cluster IX (11.29% of the total LAB isolates) were identified as *Enterococcus faecalis* with 100% identity to EzBioCloud sequences. The isolates of clusters X and XII (4.83% of the total LAB isolates) were identified as *Lactococcus lactis* with 99.20-99.33% similarity to EzBioCloud sequences. The isolates of cluster XI (6.45% of the total LAB isolates) were identified as *E. durans/faecium* with 99.45% similarity to EzBioCloud sequences.

## 4. Discussion

The technology of curdled milk in the Central region of Burkina Faso is similar to that of the South-West region presented by [2], except for the duration of the fermentation. In the present study, the fermentation lasted in a maximum of 48 h compared to that reported by [2], which lasted longer (59 h). This may be explained by the period during which the production monitoring was carried out and the processing environment. Indeed, as reported by [12], high ambient temperature lead to a quick acidification of milk, accelerating the fermentation than low ambient temperature.

The average pH value obtained for raw milk ( $6.47 \pm 0.02$ ) is closed to that found by [13] which was 6.50, but is below the pH indicated by [14] which is between 6.6 and 6.8. This low pH value may be due to an early fermentation of the raw milk due to non-immediate refrigeration after milking. The pH found is also lower than that reported by [15] which was  $6.59 \pm 0.30$ . However, it is higher than the pH found (5.9-6.2) for raw milk collected in pastures of Daloa town in Côte d'Ivoire by [16]. Low mean pH was observed for curdled milk samples ( $4.34 \pm 0.10$ ) while high acidity was observed. This important drop in pH and increase in acidity in curdled milk compared to raw milk is related to the fermentation that takes place during the manufacture of this food. Indeed, LAB produce organic acids, mainly lactic and acetic acids, which induce a lowering of pH [17,18]. The average curdled milk pH found is within the normal pH range (4 - 4.6) indicated by [14]. This pH is also comparable to the pH obtained by [4] which was  $4.37 \pm 0.24$  and [2] which was  $4.3 \pm 0.08$ .

The mean value of total dry matter content of raw milk ( $7.85 \pm 2.5\%$  w/w) is lower than the normal average value of 12.8% w/w indicated by [14]. This value is also lower than those obtained by [13] and [19] which were 11.7% w/w and between 9% and 12% w/w, respectively. The increase observed for the curdled milk dry matter could be due to the multiplication of fermenting microorganisms. Indeed, during fermentation, lactic acid bacteria use milk lactose to produce lactic acid. This acidification generates a precipitation of the proteins of the milk at isoelectric pH leading to the coagulation of the milk, this in combination with the release of various other metabolites [14,20].

The protein content of raw milk was below the FAO standard of 34 g/L [14]. This content has increased with the fermentation and may be due to the increase in cell biomass [21]. The average value of the fat content of raw milk is in line with that of [14] and [21]. However, this value is lower than that found by [22] which was 38 g/L.

The decrease in the fat content of the curdled milk samples could be explained by the fact that during the fermentation, certain strains of lactic acid bacteria possessing lipases, hydrolyzed the lipids present in the milk in short chain of fatty acids. These fatty acids will be precursors of aromatic compounds such as methyl ketone, thioesters and lactones [23].

In this study, an increase in the phosphorus content was observed during the fermentation of raw milk into curdled milk. This increase could be due to the decrease in the pH of the milk during the fermentation causing a solubilisation of phosphorus. Indeed, [24] showed that a lowering of pH leads to an increase in the content of phosphorus. The average value of iron content of the milk samples is in agreement with the value reported by [14] and that found by [25]. Iron content in cow milk is generally related to various factors such as race, diet, stage of lactation, etc. There was a decrease in the iron content during the fermentation certainly due to the metabolic activity of lactic acid bacteria. Concerning calcium, the average value of the raw milk samples is closed to that reported by [25] which was 1.25 g/L. There was an increase in the calcium content after the fermentation. This could be explained by the acidification of the milk during fermentation. Indeed, it has been proven that a drop in the pH of the milk leads to an increase in the soluble calcium content [24]. The decrease in zinc content during fermentation is probably due to the metabolic activity of fermentative strains that use these minerals for their growth.

The mean total AMB counts for raw milk samples ( $1.9 \pm 1.7 \cdot 10^7$  CFU/mL) exceeds the microbiological standard applicable to raw milk ( $5 \cdot 10^4$  CFU/mL) set by [26]. This high microbial load in the raw milk may arise from the non-hygienic practices used in the farm or the health status of the animal. [27], have demonstrated for example that containers used during milking and in the manufacture of fermented milk can cause contamination of the milk as well as the final product.

Total mesophilic bacteria counts higher than  $10^5$  CFU/mL in raw milk were reported by other authors [15,28,29]. An increase of AMB was observed in the curdled milk. According to [43], the combined effect of temperature, unsanitary environmental conditions, associated with the absence of the cold chain creates

conditions particularly favorable to the development of microorganisms.

The mean load ( $3.3 \pm 3.5 \cdot 10^5$  UFC/mL) of LAB in raw milk respect the limit ( $10^6$  UFC/mL) set by [30]. [4], however recorded higher LAB counts in raw milk with values of  $1 \pm 0.8 \cdot 10^8$  UFC/mL. The important increase of LAB count in curdled milk is due to the fermentation. This curdled milk LAB concentration is similar to that found by [20] and [16].

The yeasts and molds counts obtained in the present study for raw milk as well as for curdled milk are comparable to those reported by [13] and [4]. A high number of yeasts was also recorded by [12] and [2] in *nunu* and *lait caillé*, two spontaneously traditional fermented milk product from Ghana and Burkina Faso, respectively. The high number of yeasts suggests that yeasts are able to multiply in the milk and may result in spoilage or, conversely, in enhancement of the flavor of the fermented milk [31]. Several other studies demonstrated that yeasts constitute a significant part of the microflora of traditional fermented dairy products [32,33,34]. A possible interaction between yeasts and LAB during the fermentation process has been suggested by [32]. 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. Enterobacteriaceae counts from raw milk and curdled milk were higher than the acceptable limit of  $10^2$  CFU/mL for dairy products [26]. A high prevalence of Enterobacteriaceae has also been reported by [34] and [35] in the study of traditional fermented dairy products from Kenya. The high level of Enterobacteriaceae in the milk samples in this study can be explained by the poor hygienic conditions of production. Moreover, the manufacturing practices did not include pasteurization and relied on spontaneous fermentation, which are major risk factors that enable various microorganisms, including potential pathogenic microorganisms, to grow during the fermentation [2,36]. Thus, the occurrence of Enterobacteriaceae in the milk products may pose a risk to consumers.

In the present study, the different LAB species identified in the curdled milk collected in production sites were *L. fermentum*, *L. helveticus*, *Enterococcus durans/feacium*, *Enterococcus faecalis* and *Lactococcus lactis*. The most abundant genus in the curdled milk was *Enterococcus* (62.9% of the isolated LAB), constituted of the species *E. durans*, *E. durans/feacium* and *E. faecalis*. High amounts of *Enterococcus* spp. have been reported in other fermented milk products like *leben* from Tunisia [37], *nunu* from Ghana [12] and *lait caillé* from the South-West region of Burkina Faso [2], as well as in many traditional cheeses from the Mediterranean countries [38]. In some cases they predominate with regard to lactobacilli and lactococci [12]. Only few studies have dealt with the ability of enterococci as milk acidifiers. However, it has been reported that some species of *E. faecium* and *E. faecalis* grown in camel, ovine or caprine milk could produce relatively high amounts of lactic acid [39]. In addition, certain species of enterococci have been reported to exhibit probiotic properties [38,40]. However, some species of enterococci are recognized as opportunistic pathogens, especially *E. faecalis*. Moreover, antibiotic

resistance and virulence factors have been reported for both *E. faecium* and *E. faecalis* [44]. A high rate (25.8%) of *L. fermentum* has been isolated in the curdled milk from Ouagadougou and Loubila. This species was also identified in Fulani curdled milk from northern Burkina Faso in previous work [5]. Other studies have also reported the involvement of *L. fermentum* in the fermentation of several fermented dairy products of African origin [34,41]. [12], identified *L. fermentum* as the predominant LAB during *nunu* fermentation. *Lactobacillus helveticus* accounted for 6.45% of our isolates. It has also been identified in the fermentation of the traditional Ghanaian fermented milk, *nunu*, [12] and in *amaruranu amaruranu*, a traditional Kenyan fermented milk [35].

About 4.83% of the curdled milk isolates were *Lactococcus lactis*. [5], also identified strains of *Lactococcus lactis* in Fulani curdled milk samples from Northern region of Burkina Faso. The involvement of *Lactococcus lactis* in the fermentation of traditional fermented milks has also been reported in Tanzani [42], and South Africa [33]. In *lait caillé* from South-West region of Burkina Faso, *Lactococcus lactis* was recently identified as the predominant LAB at the end of the fermentation [2]. The variation of microbial biodiversity of traditional fermented milks is influenced by the climatic region and processing [5].

## 5. Conclusion

The present study allowed to describe the traditional process of curdled milk production in the Central region of Burkina Faso, in particular in the communes of Ouagadougou and Loubila. Curdled milk is obtained by a simple artisanal technology during which raw milk collected in calabashes or plastic containers undergoes a spontaneous fermentation during 24 to 48 h without pre-heat treatment. The biochemical characterization highlighted the nutritional potential of raw and curdled milk. The processing of raw milk through spontaneous fermentation to curdled milk resulted in important modifications of the biochemical parameters. Microbiological analyses revealed high amounts of AMB, LAB, Yeasts and molds and Enterobacteriaceae in raw milk as in fermented milk. The main LAB species identified in curdled milk were *L. fermentum*, *L. helveticus*, *Lactococcus lactis*, *E. durans/feacium* and *E. faecalis*. The high level of Enterobacteriaceae and Enterococcus spp. in the products pointed out poor microbiological quality indicating a potential risk for consumers. It is therefore necessary to train the producers on good manufacturing practices. The selection of starter cultures from the dominant LAB identified based on their technological properties for controlled production of curdled milk may also contribute to enhance the quality of the product.

## Conflict of Interest

The authors have no competing interests.

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