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Evaluation of the production technologies and the microbial and physico-chemical qualities of curdled milk produced in Benin

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ABSTRACT

Objectives: The aim was to identify the different production technologies, the hygiene of the producers and the microbiological and physico-chemical qualities of curdled milks produced in Benin.

Methodology and Results: Thirty-two curdled milk samples were collected from five municipalities and microbiological analyses were carried for the detection of *Salmonella sp.* . The enumeration of total microbial flora, total and faecal coliforms, *Escherichia coli*, lactic flora, sulfito-reducing anaerobic germs, *Staphylococcus aureus*, as well as yeasts and moulds using normalized methods was carried out. The pH and the titratable acidity of the samples were determined by the AOAC method (1990). Results showed that majority of curdled milk producers of Benin are Peulh women. Two types of milks (fresh cow milk and powdered milk) were used for the preparation of these curds with two different technologies (technology using powdered milk in their preparation with old curdled milk as a starter and that using fresh cow milk with endogenous starter) were used. Microbiological and physico-chemical analyses revealed that the average total microbial flora was 255±126.10^6 cfu/ml. The lactic flora, as well as yeasts and moulds flora were respectively 8.29±6.56 x10^6 cfu/ml, 12.431±20.706 x10^3 cfu/ml and 13±23 cfu/ml. Coliforms count varied from 11.313±13 x10^3 cfu/ml at 30°C to 0.983±1.228 x10^3 cfu/ml at 44°C, while the average *Escherichia coli* count was 0.34±0.89 cfu/ml. The average pH and titratable acidity of the samples were respectively 3.77±0.17 and 156.36±30.22 degree Dornic. All analysed curds were exempt of *Salmonella sp, Staphylococcus aureus* and *Clostridium spp*.

Conclusion and application of findings: The poor quality of the studied curdled milk samples poses serious health risks to consumers. Therefore, this study calls for producers' sensitisation and training on good hygienic practices for safer curdled milk production with less public health risk.

Key words: curdled milk, quality, technology, health risks, Benin

INTRODUCTION

In Sub-Sahara African countries, animal husbandry is one of the main activities after crop production undertaken for food security and livelihood (Sessou *et al*, 2013). Among livestock derived products, milk plays a major role in these communities (Katinan *et al*., 2012). Milk, a major food for populations in the arid and semi-arid regions with its high carbohydrate, lipids, vitamins and mineral contents, is often consumed raw or traditionally processed (Bezzalla and Gouttaya, 2013). Because of its high water and nutrient contents, milk undergoes a rapid deterioration under high temperatures characteristic of tropical countries (Sessou *et al*., 2013). With the absence of a cold chain, stakeholders of milk sector are obliged to develop different preservation and processing techniques that are adapted to their socioeconomic and environmental context (Kèkè *et al*., 2009). In Benin, milk is transformed into various dairy products such as butter, traditional cheese “wagashi”, yogurt and curdled milk. (Sessou *et al*, 2013). Curdled milk, obtained through fermentation, is one of the most popular and traditional fermented foods consumed in many countries (Nakasaki *et al*., 2008). It is an important food in many developing countries because of its cheaper production technology, high nutritional value and sensorial properties (Steinkraus, 1996). Curdled milk is commonly used as dessert or refreshment and its production in rural and urban areas became a very important income generating activity. However, the production remains domestic and artisanal (Katinan *et al*., 2012). The biochemical composition of curdled milk makes it prone to the growth of microorganisms (Ahmed *et al*., 2010) and therefore it is considered after raw milk, as one of the main causes of food poisonings (Gran *et al*., 2002). The assessment of the safety of curdled milk is therefore indispensable in order to protect consumers’ heath. Moreover, there is a lack of data on the microbiological and physico-chemical quality of curdled milk produced in Benin. Concerning the high demand of curdled milk for human consumption without any further treatment in Benin, a particular attention is required on the safety of this product. This study is therefore undertaken to identify the different production technologies of curdled milks, the characteristics of the producers in Benin and to assess the microbiological and physico-chemical qualities of these curds.

MATERIALS AND METHODS

Data collection: Data were collected from May to July 2014 aiming to assess the importance of production, the characteristics of producers and the different technologies of curdled milk production in Benin. The investigations were carried out in ten municipalities of six Departments of Benin whereby semi-structured questionnaires were administered to curdled milk producers and vendors. A total of 320 curd producers were enrolled in the study.

Table 1: Variables and modalities of curdled milk production

Variable	Modalities
Age group	Young, Adult
Sex	Male, Female
Education	Illiterate, Primary, Secondary
Ethnic group	Peulh, gando, dendi, bariba,fon
Department	Atacora, Borgou, Alibori, collines atlantic
Raw material	Fresh cow milk, powdered milk
Quantity of water	2 kg, 3 kg
Raw material supply	Peulh camp, super market, market
Milk heating	No, Yes
Type of ferment (starter)	Curdled milk, Yogurt
Curdling Container	Calabash, plastic metallic
Duration of fermentation	1-2 days, 3-4 days, 5-7 days
Production Unit	Peulh Camp, cafeteria

Analyses of curdled milk production technologies: To identify the different technological variants of curdled milk production in Benin, a multiple correspondences analysis was employed. The used variables are presented in Table1. The choice of the number of axis is based on the principle of maximal variance displayed by the axes. The interpreted modalities are those that are more represented with a strong contribution on each axis. To test whether the length of preservation of the curdled milk varies according to the methods of preservation and the types of product, Chi square tests were performed in contingency tables.

Sampling procedure: Sampling was conducted in accordance with the ISO 707 standards, whereby were aseptically collected from producers in sterile dark falcons of at least 125 ml in three zones of Benin such as the North (Parakou and Gogounou), the centre (Dassa) and the South (Abomey-Calavi and Cotonou). The falcons containing samples were carefully labelled with all pertaining information of the product. The lids of these falcons were sealed with adhesive paper. Before refrigeration, the entire falcon was covered with aluminium foil to prevent the occurrence of possible alterations from the light. Overall, thirty-two samples were collected with respect to the ethnic groups, the locations and the types of milk used as described in Table 2.

Table 2: Distribution of samples per location, ethnic groups and types of milk

Location	Ethnic group	Fresh cow milk	Powdered milk
	Peulh	4	-
Dassa	Gando	4	-
	Peulh	4	
Parakou	Gando		
	Other citizens		4
	Foreigners		4
Gogounou	Gando	4	
Abomey-Calavi	Other citizens		4
	Foreigners		4

Physico-chemical analyses of sampled curdled milks: The physico-chemical analyses consisted of the determination of the pH and the titratable acidity of the samples (Figure1). The pH was measured with a digital pH-meter (Inolab pH 730 WTW 82362 Wellheims, Germany) in 5 ml of curdled milk. The titratable acidity of the samples was determined by titrimetry with Dornic sodium (N/9) using the AOAC method (1990). Ten millilitres of the sample was poured in 100 ml conical flask to which was added two drops of phenolphtalein. The mixture was titrated with the aforementioned Dornic sodium and the obtained lactic acid content was determined in degree Dornic.

Microbiological analyses of sampled curdled milks: Microbiological analyses consisted of the detection of *Salmonella sp*. and the enumeration of total microbial flora, total and faecal coliforms, *Escherichia coli*, lactic flora, sulfito-reducing anaerobic germs, *Staphylococcus aureus*, as well as yeasts and moulds. The total microbial flora and the fungal flora were enumerated using respectively the NF EN ISO 6222: 1999 method on plate count agar at 30°C for 72 hours and ISO 7954:1987 on Sabouraud media with chloramphenicol at 25°C for 5 days. The lactic flora was counted on Man Rogosa Sharp media while *Staphylococcus aureus* count was done based on the NF EN ISO 6888 - 1:1999 standards on Baird Parker media at 37°C and *E. coli* count was conducted on Rapid *E. coli* media at 44°C based on the NF ISO 16649-2 method. The detection of *Salmonella spp* was performed using the NF EN ISO 6579: 2002 method while the spores of *Clostridium* were counted in tubes according to the XP V 08-061:1996 standard.

Statistical analyses: Each microbiological and physico-chemical parameter was compared to the standard reference value. The Student t test was then used to compare means where data were normally distributed, whereas Wilcoxon text was used in the opposite case. Each parameter was then compared between locations, ethnic groups and types of milk used by the analysis of variance.

RESULTS

Characteristics of the study population: About 87.9% of the investigated curdled milk producers were females, while only 12.1% were males. Majority of them (76.4%) were Peulh women followed by Gando (10.9%), Dendi (6.8%), Fon (2.8%), Bariba (0.6%) and foreign producers (2.5%). Up to 84.5% of the producers are uneducated, 13.7% had primary education, while only 1.9% had secondary education. Furthermore, the study population was constituted of 54.7% of young producers, 45% of adults and 0.3% of old producers.

Technological variants of curds production in Benin: Based on the principle of maximal percentage of cumulated variance, the first 6 axes on Figure 1 are retained as they also contain 65.4% of the total cumulated variance. Table 3 shows the modalities to be interpreted per factorial axis and Figure 1 displays the projection of these modalities on the 6 retained factorial axes. The analysis of Figure 1 revealed that majority of curdled milk producers of the Departments of Atlantic and Littoral are men of Fon ethnic group and foreigners. They use powdered milk in their preparation with old curdled milk as a starter. The powdered milk is mixed respectively with 10 or 15 l of water for 2 kg and 3 kg of powder. The containers used are plastics and their production units are cafeterias. These producers get their powdered milks from supermarkets (axis 1). However, producers of Bariba and Dendi origins do not use powered milk from supermarkets as they commonly produce their curds from fresh cow milk (axis 2). In production units like Peulh camps, fresh milk is not pasteurised (heated) before use (axis 3). Most of Gando producers were young and come from the Department of the Alibori, whereas Peulh producers were older and come in majority from the Departments of Borgou and Atacora. Furthermore, most Gando curd producers use metallic containers. The production unit of Peulhs was the Peulh Camp and the one of Gandos was the household (axis 4). Producers of Fon ethnic group having primary education use 2 kg of powdered milk in 10 L of water with yogurt as the starter, while foreign producers, who in majority had a secondary education use 3 kg of powder in 15 L of water (axis 5). Producers of the Department of Collines (1.9%) had an intermediate education level (grade 4). They use yogurt as the starter (Axis 6).

Table 3: Axes and modalities

Axes	Modalities
Axe 1:	Man, Dendi and Bariba, Atlantic _ littoral, powdered milk, water_2kg, water_3kg, supermarket, tech_ powdered milk, Heat_not_concerned, curdled_milk, Plastic, Cafeteria
Axe 2:	Dendi and Bariba, Foreigner, Market,
Axe 3:	Length_Fermentation_24h, Length_Fermentation_48h, Heated, Not_Heated, Household
Axe 4:	Adult, Young, Gando, Peulh, Atacora, Borgou-Alibori, Metal, Peulh-Camp, Household
Axe 5:	Man, Primary, Secondary, Foreigner, Fon, 2k_water, 3 kg_water, Market, Yogurt
Axe 6:	Secondary, Borgou_Alibori, Collines, Yogurt, Household

Figure 3(d) shows the projection of producers on the factorial plan 1 and 2. This figure reveals two groups of producers: fresh cow milk users and powdered milk users. Majority of fresh cow milk users were Peulh, and come from the Departments of Borgou, Alibori and Atacora, whereas those using powdered milk are Fon, Dendi, Bariba and foreigners.

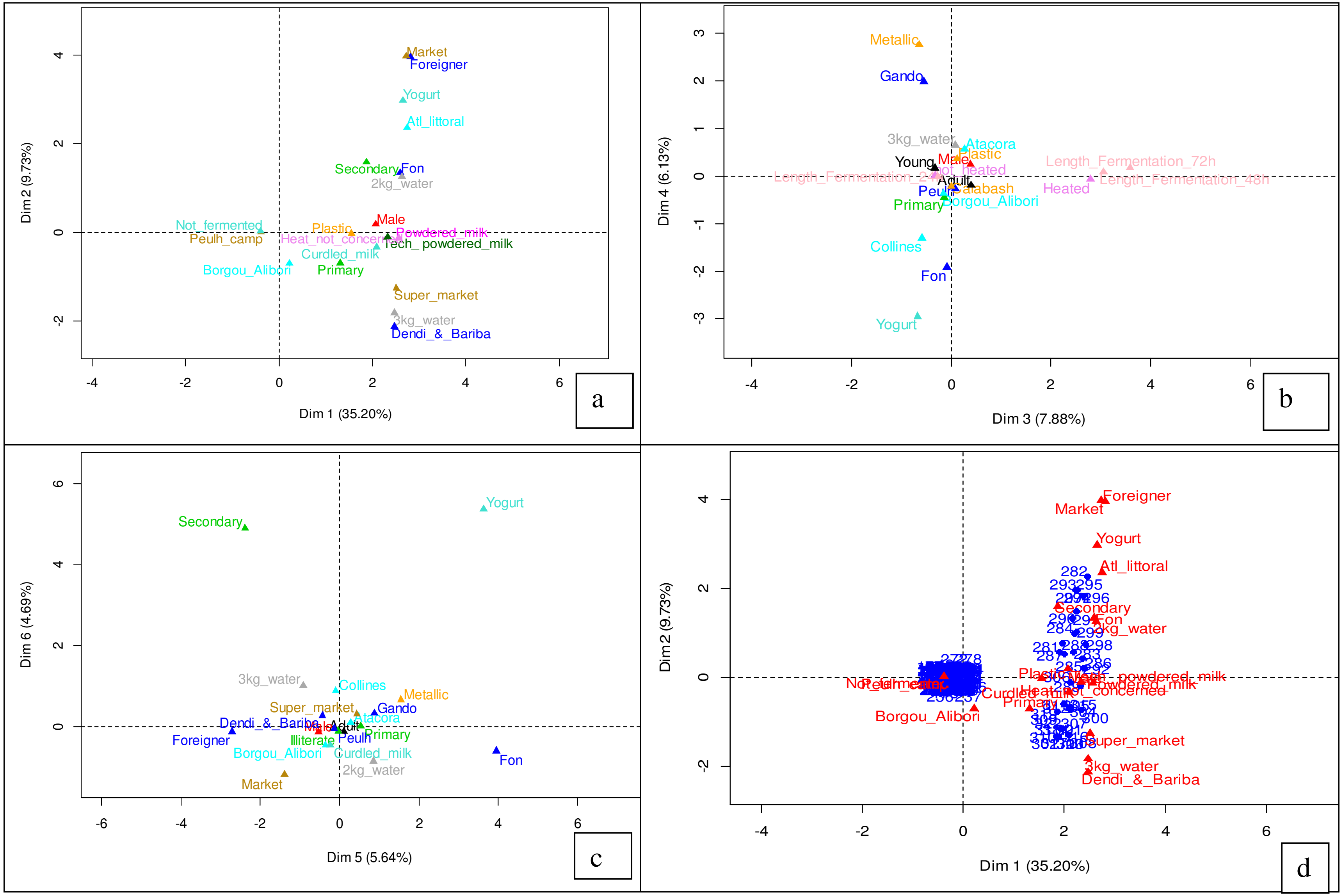

Figure 1: Characteristics of curdled milk producers

Length of preservation of curdled milks: The length of curdled milk preservation varied between producers. About 64% of them could preserve their curds for 3 to 4 days, 27% for 1 to 2 days and 8% for 5 to 7 days (Figure 2). 66.3% of producers, who use fresh cow milk, could keep their curdled milk for 3-4 days, 30.8% preserve it for 1-2 days and 2.8% keep it for 5-7 days. Among the producers who use powdered milk, 52.2% preserved their curds for 3-4 days, 6.5% for 1-2 days and 41.3% keep it for 5-7 days. The length of curdled milk preservation varied significantly with the type of milk (Chi square = 79.08, P = 0.000). Two preservation methods were used: the preservation at ambient temperature (87.0% of the producers) and refrigeration (13.0%). Figure 3 shows that the length of curdled milk preservation is longer (5-7 days), when it is kept in a refrigerator. Refrigeration was the preservation method of about 45% of producers. On the other hand, curdled milk is preserved for 3-4 days when it is kept at ambient temperature. This mode of preservation is applied by 65% of the producers. It can therefore be concluded that refrigeration can prolong the length of curdled milk preservation for 2 to 3 days more. Moreover, the length of preservation varied significantly based on the preservation methods used by the producers (Chi square = 90.29; P = 0.000).

Production Material: This study revealed that the production of curdled milk is a typically artisanal activity. The material of production is dominated by calabashes for curdling (76%) and transportation of the curds (61%). Plastic containers come in second position for curdling (21%) and transportation (20%). Metallic containers made of aluminium were used by a very few producers. However, cups were commonly used for the sale of curdled milk in cafeterias.

Quantity and source of water used for curdled milk production: Curdled milk producers who use fresh cow milk (87.0%), do use water for their production. However, the use of water is compulsory for those using powdered milk (13%) for the preparation of curdled milk. Analyses revealed that 54.8% of this type of producers uses 10 l of water for 2 kg of powdered milk and 45.2% used 15 l of water for 3 kg. Two sources of water were recorded: tap water from the National water supply authority of Benin (SONEB) and water from drilling. Nevertheless, majority of curdled milk producers (95.2%) use tap water from SONEB while 4.8% use drilling water.

Table 4: The pH of the analysed curdled milk samples

Location	Ethnic groups	Fresh cow milk (10^3)	Powdered milk	Means
Dassa	**Peulh**	3.77 ± 0.08	-	3.74 ± 0.06a
	Gando	3.72 ± 0.01	-	
	Mean	3.74 ± 0.06	-	
Parakou	**Peulh**	3.81 ± 0.07	-	3.81 ± 0.2a
	Gando	3.68 ± 0.04	-	
	Other Citizens	-	4.03 ± 0.16	
	Foreigners	-	3.61 ± 0.03	
	Mean	3.78 ± 0.09	3.82 ± 0.25	
Gogounou	**Gando**	3.76 ± 0.23	-	3.76 ± 0.23a
	Mean	3.76 ± 0.23	-	
Abomey-Calavi	**Other Citizens**	-	3.76 ± 0.15	3.76 ± 0.15a
	Foreigners	-	3.69 ± 0.1	
	Mean	-	3.76 ± 0.15	
	Overall Mean	3.76 ± 0.12	3.79 ± 0.2	3.77 ± 0.17

Values of the same column followed by the same letters are not significantly different at 5%.

Physico-chemical qualities of analysed curdled milks: The physico-chemical parameters determined in this study were the pH and the titratable acidity of the curdled milk samples. Table IV shows the different values of the pH according to the types of milk used, producers' location and their ethnic groups. The average pH of curdled milk samples was 3.77±0.17, with a minimum of 3.53 and a maximum of 4.27. However, the pH did not vary significantly between locations (F=0.5149, Prob. = 0.6736). The average pH of curds made with fresh milk was 3.76±0.12 and 3.79±0.2 for those made with powdered milk. Likewise, it did not vary significantly according to the type of milk used (F=0.7265, Prob. = 0.3973). Nevertheless, the pH varied significantly between the ethnic groups of producers, but only in Parakou city (F=12.442 Prob. =0.0000) where the pH of curdled milk from other citizens was higher than the one of producers of the other ethnic groups. These results are reliable because the average pH of curdled milk did not vary significantly from one test to another (F=0.0498 Prob. =0.8242). Table 5 showed that the average titratable acidity of the samples was 156.36±30.22°D. The lowest titratable acidity value recorded was 84°D and the highest was 220°D. Moreover, the acidity varied significantly between producers' locations (F = 14.304; P = 0.000), with the acidity of Dassa (187.88±11.67°D) being higher than those from other places. Furthermore, the average titratable acidity (165.88±24.48°D) of curds made with fresh cow milk was significantly higher than the acidity (146.83±32.69) of those made with powdered milk (F = 6.962; P = 0.010). It also varied significantly according to the ethnic groups of the producers and this difference was only recorded in Parakou (F=4.745; P = 0.011) where the highest titratable acidity values were obtained from foreigners (158.2±25.01) and other citizens (116.5±27.58). It however, did not vary significantly between Gando and Peulh producers. These results are reliable because the titratable acidity did not vary significantly between tests (F = 0.0309; P = 0.8611). For all analysed curdled milk samples, the obtained titratable acidities were beyond the minimal critical value of 120 regardless of the location, the ethnic group of the producers and the type of milk used.

Table 5: The titratable acidity of curdled milk samples (Standard value ≥120°D)

Location	Ethnic group	Fresh cow milk (°D)	Powdered milk (°D)	Mean **(°D)**
Dassa	**Peulh**	186.25±10.66	-	187.88±11.67
	Gando	189.5±13.13	-	
	Mean	187.88±11.67	-	
Parakou	**Peulh**	144±8.29	-	139.32±27.35
	Gando	141±1.41	-	
	Other citizens	-	116.5±27.58	
	Foreigners	-	158.2±25.01	
	Mean	143.25±7.17	137.35±33.33	
Gogounou	**Gando**	144.53±9.94	-	144.53±9.94
	Mean	144.53±9.94	-	
Abomey-Calavi	**Other citizens**	-	156.31±30.1	
	Foreigners	-	142.63±21.17	156.31±30.1
	Mean	-	156.31±30.1	
	Overall mean	165.88±24.48	146.83±32.69	156.36±30.22

Microbiological quality of the curdled milk samples: The microbiological analyses revealed that none of the analysed curdled milk samples contains *Staphylococcus aureus, Salmonella sp* and the spores of *Clostridium*. Results of the total microbial flora, the lactic flora, total and faecal coliforms as well as fungal flora and *E. coli* counts are shown in table 6.

Total microbial flora: Table 6 showed that the microbial load of the total flora ranged between 43 x10^{6} cfu/ml and 532.10^{6} cfu/ml with an average of 255 x10^{6}±126 x10^{6} cfu/ml. It varied significantly between locations (F = 11.074; P = 0.000), whereby curdled milks produced in Dassa had the highest total microbial flora (3681.10^{6}±108.10^{6} cfu/ml) as compared to those of the other locations. The average total flora of curdled milks prepared with fresh cow milk was 294 x10^{6}±139 x10^{6} cfu/ml and 215 x10^{6}±98 x10^{6} cfu/ml for those made with powdered milk with no significant difference (F = 2.4664; P = 0.0708). The total flora also varied significantly between the ethnic groups of producers (F = 6.5417; P = 0.000). However, this difference was only observed in Parakou (F = 3.1453; P = 0.0478) where the highest total microbial flora was obtained among Peulh producers (3331 x10^{6}±1101 x10^{6} cfu/ml). Moreover, the total flora did not vary between tests (F = 0.025; P = 0.8765), the results are therefore reliable.

Tableau I: The total microbial flora of analysed curdled milk samples

Location	Ethnic groups	Fresh cow milk ($\times 10^6$ cfu/ml)	Powdered milk ($\times 10^6$ cfu/ml)	Mean ($\times 10^6$ cfu/mL)
Dassa	**Peulh**	329±122	-	368±108
	Gando	408±81	-	
	Mean	368±108	-	
Parakou	**Peulh**	333±110	-	257±119
	Gando	95±9	-	
	Other citizens	-	217±121	
	Foreigners	-	281±92	
	Mean	273±145	249±109	
Gogounou	**Gando**	168±90	-	168±90
	Mean	168±90	-	
Abomey-Calavi	**Other citizens**	-	181±73	181±73
	Foreigners	-	178±95	
	Mean	-	181±73	
	Overall mean	294±139	215±98	255±126

As presented in Table 7, the lactic bacteria flora of all analysed curd samples varied from 0.5 x10^6 cfu/ml to 28 x10^6 cfu/ml, with an average of 8.29 x10^6±6.56.10^6 cfu/ml. Unlike for Abomey-Calavi, Dassa and Parakou where the lactic flora were similar, it varied significantly between Gogounou and Dassa and between Gogounou and Parakou (F = 4.5267; P = 0.0063). Furthermore, the average microbial load in lactic flora was 8.381 x10^6±7.44 x10^6 cfu/ml for curds prepared with fresh milk and 8.2 x10^6±5.67 x10^6 cfu/ml for those made with powdered milk. The difference was however not significant (F = 0.012; P = 0.9131). The lactic flora varied significantly between ethnic groups (F=3.2186 P = 00452) but this difference was observed only in Parakou (F=3.1196; P=0.04902), where curds prepared by Peulh producers were more contaminated (14.03 x10^6±7.81 x10^6 cfu/ml) than the others. The statistical analysis also revealed that the lactic flora did not vary from one test to another. These results are therefore reliable (F=6.6408; P=0.01236).

Table 7: The lactic flora of the analysed curdled milk samples

Locations	Ethnic groups	Fresh cow milk $\times 10^6$ cfu/ml)	Powdered milk (10^6 cfu/ml)	Mean ($\times 10^6$ cfu/ml)
Dassa	**Peulh**	9.58±5.11	-	10.55±6.68
	Gando	11.53±8.2	-	
	Mean	10.55±6.68	-	
Parakou	**Peulh**	14.03±7.81	-	9.73±6.39
	Gando	0.9±0.42	-	
	Other citizens	-	7.89±5.42	
	Foreigners	-	10.54±4.28	
	Mean	10.75±8.98	9.21±4.92	
Gogounou	**Gando**	1.68±0.71	-	1.68±0.71
	Mean	1.68±0.71	-	
Abomey-Calavi	**Other citizens**	-	7.19±6.33	7.19±6.33
	Foreigners	-	5.43±7.71	
	Mean	-	7.19±6.33	
	Overall mean	8.38±7.44	8.2±5.67	8.29±6.56

Yeasts count: Table 8 shows that the yeasts count was between 100 cfu/ml and 118.400 x10^3 cfu/ml with an average of 12.431x10^3±20.706 x10^3 cfu/ml. It varied significantly between locations (F=14.113; P = 0.0000) with Abomey-Calavi having the highest yeast count. The average yeast count was 3.469±4.992 x10^3 cfu/ml for curdled milk prepared with fresh milk and 21.394±26.087 x10^3 cfu/ml for those made with powdered milk with a significant difference (F=24.066; P = 0.0000). The number of yeasts also varied significantly between producers' ethnic groups (F=16.37; P=0.0000). This difference was mainly observed in Parakou (F=50.586; P=0.0000), where foreigners' curds were the most contaminated ones (25.488±8.635 x10^3 cfu/ml) followed by curds from Peulh producers (11.333±7.393 x10^3 cfu/ml). Moreover, the yeast flora did not vary between tests (F=2.4073; P = 0.1259).

Table 8: Number of yeasts enumerated from the curdled milk samples

Locations	**Ethnic groups**	**Fresh cow milk** (**10^3 cfu/ml**)	**Powdered milk** (10^3 cfu/ml **)**	Mean **(10^3 cfu/ml)**
Dassa	**Peulh**	1.713±1.615	-	1.713±1.549A
	Gando	1.713±1.591	-	
	Mean	1.713±1.549	-	
Parakou	**Peulh**	1.1333±7.393	-	1.1871±1.75B
	Gando	0.550±0.212	-	
	Other citizens	-	1.488±1.212	
	Foreigners	-	25.488±8.635	
	Mean	8.638±7.998	13.488±13.751	
Gogounou	**Gando**	1.813±0.720	-	1.813±0.720AB
	Mean	1.813±0.720	-	
Abomey-Calavi	**Other citizens**	-	29.300±32.924	29.300±32.924C
	Foreigners	-	16.425±13.458	
	Mean	-	29.00±32.924	
	Overall Mean	3.469±4.992	21.394±2.,087	12.431±20.706

Moulds count: Table 9 shows that the average number of moulds in the curdled milk samples was 13±23 cfu/ml, with 0 cfu/ml being the lowest count while the highest mould count was 80 cfu/ml. The average mould count did not vary significantly between locations (F = 2.4073; P=0.1259). Although the count was grossly high in Dassa, the difference was not significant. Curdled milks made with fresh cow milk had an average mould count of 23±28 cfu/ml while 3±10 cfu/ml was the average mould count from curds prepared with powdered milk. However, this gross difference between the two types of milk was not significant (F=2.4073; P = 0.1259). Likewise, the mould count did not vary significantly between the ethnic groups of the producers and remained constant from one test to another. These results are can therefore be judged reliable (F=0.4; P = 0.5336).

Table 9: The mould count of the curdled milk samples

Location	**Ethnic group**	**Fresh cow milk**	**Powdered milk**	Mean
Dassa	**Peulh**	35±28	-	29±29
	Gando	24±30	-	
	Mean	29 ±29	-	
Parakou	**Peulh**	3±5	-	3±7
	Gando	5±7	-	
	Other citizens	-	4±11	
	Foreigners	-	0±0	
	Mean	4±5	2±8	
Gogounou	**Gando**	28±33	-	
	Mean	28±33	-	
Abomey-Calavi	**Other citizens**	-	4±12	

	Foreigners	-	9±16	4±12
	Mean	-	4±12	
	Overall mean	23±28	3±10	13±23

Total coliforms count Table 10 shows that the average number of coliforms at 30°C was 11.313 x10^3±13 x10^3 cfu/ml with a minimum of 10^3 cfu/ml and a maximum of 72 x10^3 cfu/ml. The coliforms counts were statistically similar regardless of the locations of producers (F=1.6054 P = 0.1976). The average total coliforms count at 30°C was 13.219 x10^3±13.013 x10^3 cfu/ml for curdled milk prepared with fresh cow milk and 9.406 x10^3±12.909 x10^3 cfu/ml for powdered milk. This difference between the types of milk used was significant (F=6.6968; P=0.01202). Moreover, the coliform counts varied significantly according to the ethnic groups of producers (F=2.4741; P=0.02284). The difference was only observed in Parakou (F=3.0703; P = 0.05134) where Peulh producers had the highest coliform count (19.667.10^3±25.835.10^3 cfu/ml) followed by the other citizens (18.875 x10^3±21.996 x10^3 cfu/ml). However, a significant variation was observed for the coliform counts from one test to another (F=7.6625 P=0.007425).

Tableau 10: Total coliforms counted in curdled milk samples

Location	Ethnic group	Fresh cow milk (10^3 cfu/ml)	Powdered milk (10^3 cfu/ml)	Mean (10^3 cfu/ml)
Dassa	**Peulh**	9.125±7.080	-	11.938±9.066
	Gando	14.750±10.389	-	
	Mean	11.938±9.066	-	
Parakou	**Peulh**	19.667±25.835	-	12.958±18.793
	Gando	5.500±0.707	-	
	Others citizens	-	18.875±21.996	
	Foreigners	-	3.875±3.314	
	Mean	16.125±22.800	11.375±17.056	
Gogounou	**Gando**	12.875±6.357	-	
	Mean	12.875±6.357	-	
Abomey-Calavi	**National**	-	7.438±6.723	7.438±6.723
	Foreigners	-	9.750±6.923	
	Mean	-	7.438±6.723	
	Overall mean	13.219±13.013	9.406±12.909	11.313±13.000

Faecal coliforms or thermotolerants: Table 11 shows that the average faecal coliforms count was 0.983 x10^3±1.228 x10^3 cfu/ml, with a minimum of 10^3 cfu/ml and a maximum of 6.20 x10^3 cfu/ml. They varied significantly between locations (F=14.107; P=0.000) with Gogounou harbouring the highest faecal coliforms count as compared to other locations (3.3 x10^3±1.693 x10^3 cfu/ml). The average faecal coliforms count was 1.169 x10^3±1.543 x10^3 cfu/ml for curdled milk prepared with fresh cow milk and 0.797 x10^3±0.781 x10^3 cfu/ml for powdered milk. Statistical analyses revealed that this difference between the counts from the two types of milk was not significant (F=0.1755; P =0.6767). Nevertheless, this quantum varied significantly between ethnic groups (F=8.5126; P=0.000). This was mainly observed among the ethnic groups of Abomey-Calavi (F=7.2586 P=0.01745). Moreover, faecal coliforms count did not vary from one test to another. These results are therefore reliable (F=1.1861 P = 0.2803).

Table 11: Faecal coliforms count from the curd samples

Locations	Ethnic groups	Fresh cow milk (10^3 cfu/ml)	Powdered milk (10^3 cfu/ml)	Mean (10^3 cfu/ml)
Dassa	**Peulh**	0.400±0.239	-	0.306±0.205
	Gando	0.213±0.113	-	
	Mean	0.306±0.205	-	
Parakou	**Peulh**	0.533±0.327	-	0.713±0.662
	Gando	1.450±1.344	-	
	Other citizens	-	0.425±0.276	
	Foreigners	-	0.950±0.830	
	Mean	0.763±0.717	0.688±0.656	
Gogounou	**Gando**	3.300±1.693	-	
	Mean	3.300±1.693	-	
Abomey-Calavi	**Other citizens**	-	0.906±0.896	0.906±0.896
	Foreigners	-	1.450±1.000	
	Mean	-	0.906±0.896	
	Overall mean	1.169 ± 1.543	0.797±0.781	0.983±1.228

***E. coli* count:** Table 12 shows that the average *E. coli* count was 0.34±0.89 cfu/ml, with a minimum of 0 cfu/ml and a maximum of 5 cfu/ml. The count did not vary significantly according to producers' locations ($F = 1.1861$; $P = 0.2803$). The average E. coli count was 0.06±0.25 cfu/ml for curdled milk prepared with fresh cow milk and 0.63±1.18 for powdered milk. This difference between the types of milk used was not significant ($F=1.1861$; $P=0.2803$). However, the count varied significantly between producers' ethnic groups ($F=8.5126$; $P=0.000$) and this was recorded among the ethnic groups of Abomey-Calavi ($F=7.2586$; $P=0.01745$). Furthermore, E. coli count did not vary from one test to the other. These results are therefore reliable.

Table 12: *E. coli* counts of the analysed curdled milk samples

Locations	Ethnic groups	Fresh cow milk (cfu/ml)	Powdered milk (cfu/ml)	Mean (cfu/ml)
Dassa	**Peulh**	0.25±0.46	-	0.13±0.34
	Gando	0±0	-	
	Mean	0.13±0.34	-	
Parakou	**Peulh**	0±0	-	0.42±0.83
	Gando	0±0	-	
	Other citizens	-	1.25±1.04	
	Foreigners	-	0±0	
	Mean	0±0	0.63±0.96	
Gogounou	**Gando**	0±0	-	0±0
	Mean	0±0	-	
Abomey-Calavi	**Other citizens**	-	0.63±1.41	0.63±1.41
	Foreigners	-	0.25±0.46	
	Mean	-	0.63±1.41	
	Overall Mean	0.06±0.25	0.63±1.18	0.34±0.89

DISCUSSION

The present study revealed that the pH of the curdled milk samples varied between 3.53 and 4.27 with an average of 3.77±0.17. This average pH value is similar to the one reported by Mirghani (1994) (3.9-4.0) during his study on fermented camel milks and that of Hamza (1996) (3.8-4.1) for curdled milks in Niger. These values are also similar to those obtained by Barat *et al*. (2014) (3.8-4.5) for fermented Camel milk in Iran. They are however lower than those reported by Sulieman *et al*. (2006) for curdled milk and those of Julius *et al*. (2004) (4.17 to 5.16) in fermented milk in Kenya. The pH of this study are also lower than those described by Katinan *et al*. (2012) for curds in Ivory Coast and Dieng (2001) for curds in Senegal that varied respectively from 4.84±0.25 to 5.02±0.16 and 3.96 to 4.9. These variations can be due to the curdling technology and the environment of production, as well as the length of preservation of the products. Overall, the pH of the samples is in conformity with the AFNOR standard (JORF, 1980). The average titratable acidity recorded in this study was 156.36±30.22 degree Dornic. This value is greater than to the one described by Barat *et al*. (2014) for fermented Camel milk in Iran that varied from 29.8 to 36. It is also higher than the one reported by Katinan *et al*. (2012) for curds in Ivory Coast that varied from 101.77±16.8 to 113.53±17.48 and the one of Hamza (1996) (75-100) in curds from Niger. Furthermore, the titratable acidity reported in this study is higher than the one of Biatcho (2006) in Senegal that varied from 45 to 100. The decrease of pH was accompanied by the increase of the titratable acidity of the samples. The different values recorded for the titratable acidity in this study are in accordance with the French standard reported by Katinan *et al*. (2012). These results confirm the acidic characteristic of curds. The acidity of the analysed curds can also be attributed to their high lactose content and the high lactic flora of the samples that fermented the lactose into important quantity of lactic acid. According to Fadela *et al*. (2009), the titratable acidity of curdled milk samples increases with their lactic bacteria load. The decrease of pH alongside the increase of titratable acidity of the samples can also be due to the length of fermentation of the product. In fact, Conté (2008) discussed that the acidity of curdled milk increases with the length of milk fermentation. The average total microbial flora of the analysed curds produced in Benin was 255±126 x10^6 (8.40 ± 8.1 log10 cfu/ml). This microbial flora was more or less constituted of lactic bacteria and yeasts and was higher than the one recorded in Sudan by Abdel Gadir *et al*. (2005) which was 5.08 – 6.64 log10 cfu/ml in fermented milk samples. It is also higher than that of Barat *et al*. (2014) in fermented camel milk from Iran that was about 5.78 log10 cfu/ml. However, the total flora reported in this study is lower than the one of Katinan *et al*. (2012) in Ivory-Coast that varied from 8.20±9.1 x10^4 cfu/ml to 1.47±0.98 x10^5 cfu/ml for curds and similar to those obtained by Julius *et al*. (2004) for fermented milks in Kenya (8.1 log10 cfu/ml). The average total and faecal coliforms counts of the present study were respectively 11.313 x10^3±13 x10^3 cfu/ml (4.05±4.11 log10 cfu/ml) and 0.983±1.228 x10^3 (2.99±3.08 log10 cfu/ml). These values are lower than those reported in Malaysia (17.104 cfu/ml) by Fook *et al*. (2004) and those reported in Ivory Coast (2.80±4.86.10^4 cfu/ml to 7.12±6.32 x10^4 cfu/ml) by Katinan *et al*. (2012). Furthermore, the present study revealed that the average E. coli count was 0.34±0.89 cfu/ml which is quite lower than those of Fook *et al*. (2004) (6.8 x10^3 cfu/ml) and Katinans *et al*. (2012) that varied from 3.80±7.2 x10^3 cfu/ml to 2.41±4.16 x10^4 cfu/ml. Studies on fermented dairy products in Zimbabwe demonstrated that E. coli and coliforms varied from 2 to 8 log10 cfu/ml (Gran *et al*., 2002b, c). These high coliform counts in curdled milk samples can be attributed to the poor hygiene practices during the preparations. The presence of total and faecal coliforms and particularly E. coli is the samples indicates a poor hygiene and reflects a faecal contamination of the curds and implies as well the presence of other enteric bacteria in the samples (El-Zyney *et al*., 2007). Furthermore, the presence of coliforms and other microorganisms in the curds shows a possible bacterial cross-contamination by the utensils and the water used in the preparation (Chye *et al*., 2004). The fact that up to 84.5% of curd producers were uneducated and none of the producer received food hygiene training could explain the high level of coliforms and other microbes in the processed curds. The high level of microbial contamination of the curds could therefore be related to a number of factors such as lack of personal, environmental and sanitary hygiene and use of contaminated utensils. According to Gran *et al*. (2002), plastic utensils used during the preparation of curdled milk could have a high level of coliforms contamination due to the many cracks found inside these containers. Majority of curd producers use calabashes and polyethylene plastic containers for curdling. The inner surface of these containers could have a number of cracks that could harbour many germs if these containers are not thoroughly washed or rinsed with potable water. Yet, the majority of curd producers in Benin are peulh people who live in rural areas where potable water is scare. The presence of *Escherichia coli* in some samples could imply the presence of other pathogenic enteric microorganisms (Gran *et al*., 2002a, bc). The low E. coli count of the samples of this study could be explained by the low pH of the samples and their high concentration in lactic acid. It could also be due to the presence of natural antibacterial compounds active on *Escherichia coli* isolates in the samples. According to Bezzalia and Gouttaya (2013), curdled milk has the capacity to inhibit the growth of pathogenic micro-organisms because it contains enzymes that have antibacterial and antiviral properties. The average values of lactic bacteria and yeasts count for the curdled milks analysed in this study were respectively 8.29±6.56 x10^6 (6.91±6.81 log10 cfu/ml) and 12.431±20.706 x10^3 (3.09±3.31 log10 cfu/ml). The values of lactic flora of this study were lower than those of Julius and al. (2004) in fermented milk samples (8.0 log10 cfu/ml) and the values of yeasts count of this study were lower than those reported by Julius *et al*. (2004) that varied between 4.24 and 7.44 log10 cfu/ml, with an average of 6 log10 cfu/ml. The average mould count in this study (1.11±1.36 log10 cfu/ml) was lower than those of Barat *et al*. (2014) for fermented camel milk in Iran (4.11-3.9 log10 cfu/ml). The high microbial loads in lactic bacteria, as well as in yeasts are probably due to bacterial growth during the fermentation of the raw milk (Gran *et al*. (2002). The presence of yeasts and lactic bacteria constitute an important asset for a food product. In many dairy products, yeasts contribute significantly to the development of the characteristics of milk (Beresford *et al*., 2001). With their proteolytic and lipolytic activities, yeasts can generate aroma precursors (free amino acids and fatty acids) that can lead to a significant improvement of the product's flavour (Vasdinyei and Deak, 2003). Yeasts are also capable of increasing the nutritional value of food and therefore known for the improvement of human health. The detoxifying action of mycotoxins present in yeasts was also reported (Greppi *et al*., 2013). The "Generally Recognized as Safe" lactic bacteria play a major role in the acidification of milk and curd and have a fundamental role in the microbial balance of milk (Tormo, 2010). They constitute efficient natural preservatives capable of conserving the hygienic qualities of food, because of their inhibitory properties on pathogenic microorganisms (Caridi *et al*., 2003). Lactic bacteria produce many metabolites with antimicrobial properties such as organic acids, hydrogen peroxide, carbon dioxide, reuterin, diacetyle and bacteriocins (Dortu and Thonart, 2009). Moreover, the present study showed that no Salmonella isolate was recovered from the samples. These results are similar to those of Barat *et al*. (2014) on fermented camel milks in Iran but contrary to those obtained by the same authors where Salmonella were present in eight samples of fresh camel milk. The absence of Salmonella in the samples constitutes also an important asset to these products that are highly consumed in Benin. Salmonella sp are responsible for many serious food poisonings like typhoid and paratyphoid fevers (Farougou *et al*., 2012). Furthermore, this study revealed the absence of Staphylococcus aureus in all analysed samples. These results are contrary to those of Abdel *et al*. (2005), Barbour *et al*. (1985), Chaffer *et al*. (2000), Semereab (2000), Tuteja *et al*. (2007) and Elgadi *et al*. (2008) who reported the presence of Staphylococcus spp in different samples of camel milk. The absence of S. aureus and Salmonella in the samples of this study could be due to high level of acidity of the samples and their low pH. The high acidity of these samples could inhibit the growth of *S. aureus* and *Salmonella* isolates as opposed to the samples studied by other authors. It could also be due to the possible production of antibacterial substances by the different lactic floras that prevent the survival of *S. aureus* and *Salmonella* isolates. According to Tamagnini *et al*. (2006), the production of acid and antibacterials in a food product by lactic bacteria inhibit the growth of pathogens by lowering the pH of the product.

CONCLUSION

Food security is a major priority in all public health programs. The study was dedicated to the appreciation of the physico-chemical and microbiological qualities of curdled milk produced in Benin. It revealed that the microbial loads of coliforms found in the curd samples in Benin are very high, and shows a failure in hygiene practices during the processing of raw materials, as well as the curdled milks. The analysed samples were exempt of Salmonella and S. aureus. The high titratable acidity of the samples correlated positively with their low pH. The analysed curdled milk samples are not of satisfactory quality with respect to safety standards. Therefore, the consumption of these products is not without danger to the consumer. Good hygienic practices and good manufacturing practices should be improved in the places of curds production. This study calls for other research to identify the different indigenous useful microbial strains of these milks that can be used as starters and probiotics.

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