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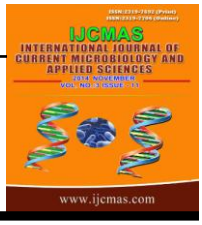

Original Research Article

Effect of manufacturing practices on the microbiological quality of fermented milk (*Pendidam*) of some localities of Ngaoundere (Cameroon)

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Keywords

Fermented milk, *Pendidam*, Manufacturing practices, Microbiological quality, Shelf life.

A B S T R A C T

It has been known for years that fermented milk products represent a way to increase shelf life of fresh milk. This study aim to evaluate the effect of manufacturing practices on the microbiological quality of fermented milk (*pendidam*) produced in Ngaoundere. An investigation on manufacturing practices was carried out in five localities of production of *pendidam*. The results indicate that two processes are mainly used to produce *pendidam* and they differ by the fact that one of them included a unitary operation of pasteurization. Based on the results of the investigation, three localities were selected for sampling of *pendidam* in order to carry out some chemical and microbiological analysis. Results of these analysis show that, the process which included pasteurization contribute to reduce significantly ($p < 0.05$) the microbial load ($9.8 \pm 0.14 \times 10^6$ cfu/mL at J_1 to $1.60 \pm 0.05 \times 10^5$ cfu/mL at J_4 for PM1 sample manufactured with process A) of the *pendidam*. However, the microbial load of all samples was higher than standards recommended for fermented milk. This study shows that unitary operation of pasteurization during manufacturing practices of *pendidam* ameliorate its microbiological quality and consequently could increase its shelf life.

Introduction

Milk from ruminant animals is an excellent source of nutrients (Aggad *et al.*, 2009; Ahmed *et al.*, 2010) and has been an important component of the human diet for thousands of years (Kennelly *et al.*, 2005). It harbors a natural microbial flora and/or other micro-organisms, which vary within a wide range of products available on the market. Because of its highly nutritive value and low acid content (Doris *et al.*, 2006), fresh milk is more sensitive to contamination and proliferation of microorganisms leading to spoilage of the product and it also constitutes a risk for consumers.

Deterioration of milk is mainly due to moulds, yeasts, bacteria and some of these

microorganisms can be very harmful for consumers (Ahmed *et al.,* 2010). To this end, processing technologies based on the transformation of fresh milk into derived products such as cheese, dairy desserts, ice cream and powdered milk have been introduced. Because of its low cost, fermentation has been used for thousands of years in the world as an interesting method of preservation of fresh milk (Gerrit, 2003). Fermented milk is one of the most popular traditionally consumed dairy products in many countries (Nakasaki *et al.,* 2008). In Africa, fermented milk is widespread and consumed by many populations (Duteurtre, 2003). Fermented milk are also of great significance based on their therapeutic value (like in the alleviation of lactose intolerance), social value and they are also used as a means of income generation (Beukes *et al.,* 2001).

In Cameroon and at the Adamaoua' region precisely, two types of fermented milk are mainly produced: *pendidam* and *kindirmou* (Libouga *et al.,* 2005). *Pendidam*, a fermented milk product, manufactured by skimmed, heated and fermented fresh milk, is highly consumed by the population of all strata of society whatever their age and origin (Essomba *et al.*, 2005).

However, the studies carried out by Tiku *et al.* (1999) have reported the poor microbiological quality of *pendidam* collected from the Adamaoua and the western highlands of Cameroon because of its high coliforms content. In the same way, studies carried out by Libouga *et al.* (2005) and Dongmo (2008) reported the poor microbiological quality of fermented milks sold in Ngaoundere. They also indicated a great variability of manufacturing technology of these artisanal fermented dairy products among the producers. Based on the fact that the manufacturing processes have an influence on the final fermented product, an outstanding question remains on the effect of the manufacturing technology on the microbiological quality of such dairy products.

The objective of this study is to evaluate the effect of manufacturing practices on the microbiological quality of fermented milk (*pendidam*) produced in Ngaoundere.

Materials and Methods

Inquiry

Investigation was carried out in March 2013. Five localities of Ngaoundere in Adamaoua' region of Cameroon that produce *pendidam* were chosen: Wakwa, Bini, the market of Dang, the small and great market of Ngaoundere town. In these localities, producers of *pendidam* were targeted for the investigation and were selected randomly.

The aim of the investigation was to collect information about socio-economic factors and other parameters which affect the post-production losses of *pendidam*. Thus, a questionnaire have been prepared and submitted to producers. It concerns: the source of raw milk, the manufacturing process of *pendidam*, and the methods of conditioning the end product, the mode of conservation and the shelf life of the product. The software Sphinx Plus^2 Lexica enables us to carry out the cards of investigation and to analyze the obtained results.

Sampling of *pendidam*

Three localities based on the results of the investigation were selected for sampling of *pendidam*: the market of Dang (PM_1), the small market of Ngaoundere town (PM_2) and Wakwa (PM_3). In each locality, the

number of samples was varied with the manufacturing process. Samples of 1 L of *pendidam* were collected in sterile flask from local producers at first day of the manufacturing (J_1) and stored in an icebox containing ice blocks during the transport.

Chemical and microbiological analysis of the samples of *pendidam*

These analyses were carried out to evaluate the effect of the various manufacturing practices on the chemical and microbiological quality of *pendidam*.

Chemical analysis

The chemical analysis of *pendidam* samples were based on the determination of pH and titratable acidity. Dornic acidity was determined by titrating 10 mL of *pendidam* samples with 0.1N NaOH in a burette after addition of 3 drops of 0.5% phenolphthalein indicator and the determination of pH was done by dipping a pH meter (pH HACH HQ 40d) in 5 mL each of *pendidam* samples until readings were stable.

Microbiological analysis

For these analysis, the choice of microorganisms to quantify were done according to European standards on fermented milk in 2005 (EC N 2073/2005) and the works carried out by Libouga *et al.* (2005) and Dongmo *et al.* (2008) on fermented milks of Ngaoundere. The quantified germs were: total coliforms, fecal coliforms, *Staphylococcus aureus*, fecal *Streptococci*, moulds and yeasts, sulphite-reducing *Clostridium*, *Salmonella* spp. and the total aerobic flora.

Preparation of samples

Aseptically, 25 mL of each sample were added into 225 mL of sterile 0.85 % NaCl solution (diluent) and mixed thoroughly. Serial dilutions (10^{-1} to 10^{-6}) were performed.

Plate counting

0.1 or 1 mL samples of appropriate dilutions were spread or poured on Petri dishes containing the following media to count group or specific micro-organisms: (i) Plate Count Agar for total viable count (Liofilchem Diagnostici, Italy), (ii) Slanetz and Bartley agar (Liofilchem Diagnostici, Italy) for fecal *Streptococci*, (iii) Chapman agar (Liofilchem Diagnostici, Italy) for *Staphylococcus aureus*, (iv) SS agar (Liofilchem Diagnostici, Italy) for *Salmonella* spp. (for (i), (ii), (iii) and (iv), incubation was done at 37°C for 48 h); (v) EMB agar (Liofilchem Diagnostici, Italy) for total and fecal coliforms, incubated at 37°C and 44°C for 48 h respectively, (vi), Potato Dextrose Agar (Liofilchem Diagnostici, Italy) for yeasts and moulds, incubated at 25°C for 3–5 days, (vii) Tryptone Sulfite Neomycin agar (Liofilchem Diagnostici, Italy) for sulphite-reducing *Clostridium*, incubated in aneorobiosis at 37°C for 48 h. The results of counting were expressed as colony forming unit per mL of *pendidam* (cfu/mL). All the tests were carried out in triplicate.

Statistical analysis

The data were noted as mean ± standard deviation and analyzed using STATGRAPHICS Plus 5.0, Sigma Plot 11.0 and Excel computers software.

Results and Discussion

Analysis of the investigation

28 producers were investigated in the five localities of Ngaoundere where *pendidam* was sampled. The results obtained show that

60.7 % of women are involved in the production of *pendidam* and among them, 39.3 % are aged of 50 years or above. Within these producers, 25 % are housewives, 37.7 % are traders and 60.7 % have an academic qualification lower than first school leaving certificate.

Among the localities investigated, Wakwa and the market of Dang have scored the greatest numbers of producers (35.7 and 28.6 % respectively). Producers of *pendidam* with high score were also recorded in the locality of Wakwa by Mahbou (2009). The higher score obtained in these localities could be explained by the fact that breeding and agriculture are the main activities of the population.

Concerning the consumption of *pendidam*, the results show that 25 % of the investigated populations consume it every day while 10.7 % consume it only twice a week. In the same way, 60.7 % consume *pendidam* directly after manufacturing and 39.3 % in association with gruel. Concerning the choice of *pendidam* as milk product, 60.7% preferred it because of its availability, among them 46.4 % because of it low price and 53.6 % because it is appreciated. The results obtained in this study concerning consumption of *pendidam* were in accordance with those reported by Essomba *et al.* (2005) who showed that, *pendidam* is a traditional fermented milk consumed in various forms by all strata of the population in Ngaoundere.

The results of the investigation indicate that two processes are mainly used to manufacture *pendidam* in Ngaoundere: one of them includes a unitary operation of pasteurization (Process A) (57.1%) and the other doesn't include pasteurization (Process B). The two processes of manufacturing *pendidam* in Ngaoundere are presented in Figure 1.

In the process of manufacturing *pendidam*, 100 % of producers use as raw material, fresh milk which is in 60.7 % of cases bought from milk farmers, in 21.4 % cases directly milking from the cows and in the other cases obtained as a gift. 89.3 % of the producers use an older *pendidam* as starter and the others use yoghourt or lemon. As reactor for manufacturing *pendidam*, 50 % of producers use calabash while 21.4 % use plastic jogs.

Concerning the mode of conditioning and conservation of *pendidam*, 82.1 % of the producers use plastics bottles for conditioning *pendidam* after the manufacturing. Once packaged, *pendidam* are preserved at room temperature in 78.6 % of case and in 21.4 % of cases at refrigerated temperature.

The fact that *pendidam* obtained from process A of manufacturing which included pasteurization has a low microbial load than the others could be explained by the fact that pasteurization reduce the initial microbial load by killing their vegetative forms. In the same way, the long shelf life of *pendidam* conserved at refrigerated temperature than those conserved at room temperature could be explained by the fact that at refrigerated temperature the growth of microorganisms are inhibited and only psychrotrophilic microorganisms can grow.

The short shelf life on *pendidam* which is in 64.3 % of cases less than 4 days at refrigerated temperature could be explained by the low level of hygiene observed during the process. The conservation of *pendidam* at room temperature which promotes the proliferation of microorganisms could also explain the short shelf life and the post-production losses of the product.

Chemical analysis of *pendidam*

The results of the acidity of our different samples of *pendidam* are represented in Figure 2 (A and B). It can be deduced from Figure 2 (A) that all samples of *pendidam* have a low pH value which varies from 4.38 ± 0.06 (for sample PM_3 collected at the day of manufacture) to 3.26 ± 0.05 (for sample PM_2 collected at the small market of Ngaoundere four days after the manufacture).

These low pH values in the samples could be explained by the metabolic activities of lactic acid bacteria used as ferment. They produced during the fermentation process, lactic acid which contributes to reduce the pH of the milk (Lyhs, 2002; Baliarda, 2003). Similar results have been obtained by Mbawala *et al.* (2013a; 2013b) with *pendidam* sampling in different localities of Ngaoundere. They obtained pH values of *pendidam* ranging from 3.38 ± 0.01 to 4.39 ± 0.03 and from 4.01± 0.07 to 4.47 ± 0.11 respectively. In the same way, Tiku *et al.* (1999) also reported in their studies, pH value ranging from 4.2 ± 0.62 to 3.5 ± 0.62 with *pendidam* of Adamawa region and from 4.2 ± 0.44 to 3.85 ± 0.44 with *pendidam* of western highlands of Cameroon.

For a same sample, it can be noticed from Figure 2 that the pH value decreases with time. This variation of pH value of *pendidam* sample with time could be explained by the increase of production of organic acids by lactic acid bacteria after the degradation of lactose.

Concerning the titratable acidity, it can be seen from Figure 2 (B) that the titratable acidity of *pendidam* samples varies from 93.31 ± 2.19 °D for sample PM_3 (collected at Wakwa on the day of manufacture) to 129.79 ± 1.22 °D for sample PM_2 (collected at the small Ngaoundere after four days of manufacture).

As opposed to pH values which decreased with time, titratable acidity was observed to increase with time. Similar observations have been reported by Mbawala *et al.* (2013a, 2013b) with *pendidam* sampling at different localities of Ngaoundere and by Tiku *et al.* (1999) with *pendidam* sampling at Adamawa region. In the same way, Katinan *et al.* (2012) reported that the titratable acidity of fermented milk sold at Yamoussoukro town, varied from 101.77 ± 16.8 °D to 113.53 ± 17.48 °D. On the other hand, the results obtained in the present study are different to those reported by Libouga *et al.* (2005). They found values of titratable acidity ranging from 60 to 90 °D for fermented milks (*kindirmou* and *pendidam*) sold in Ngaoundere and Garoua. The high values of the titratable acidity obtained in this study could be due to manufacturing process which are artisanal and not controlled or to the starter culture used by producer which are old compared to those used in the pilot center of Garoua (starter culture and standardized process) where the work of Libouga *et al.*. (2005) has been carried out.

Microbiological analysis of *pendidam*

In order to determine the influence of the manufacturing process and storage time on the microbiological quality of *pendidam*, analysis were carried out and the results obtained are represented in Table 1. From this table, it can be seen that the rates of contamination varied with the manufacturing process.

Total aerobic mesophilic count

Following the microbial analysis, the sample

containing the highest amount of aerobic mesophilic count was that collected at the small market of Ngaoundere town (PM_2): $2.20 \pm 0.07 \times 10^7$ CFU/mL and that which contained the least count was the sample collected at Wakwa (PM_3) : $8.40 \pm 0.14 \times 10^4$ CFU/mL. This is probably due to the manufacturing practice since, as opposed to the sample collected at the small market of Ngaoundere town, the sample collected from Wakwa included in its manufacturing process a unit operation of pasteurization which is necessary to reduce its microbial load. These results are in accordance with those reported by Jiwoua and Millière (1990) concerning the total aerobic mesophilic count of *pendidam* of Adamaoua region (10^6–10^8 CFU/mL) and by Tiku *et al.* (1999) with *pendidam* sampling at Adamaoua region (10^5–11.5×10^6 CFU/mL). These results are also similar with those obtained by Benkerroum *et al.* (1984) in Morocco for fermented milk called "iben" (10^8 CFU/mL).

However, the value of total aerobic flora obtained in this study was higher than those reported by Libouga *et al.* (2005) and Dongmo *et al.* (2008) for *pendidam* sold in Ngaoundere where they found a load ranging from 10^4 to 6.00×10^5 CFU/mL and 5.03×10^3 to 9.53×10^3 CFU/mL respectively. This difference could probably be attributed to a non respect of good manufacturing practices or good hygiene practices during the manufacturing process of ours samples. It is already demonstrated that high load in total aerobic flora in food is correlated to the risks of presence of pathogenic germs due to non-respect of good hygiene practices and good manufacturing practices in the chain of production (Aboubakar *et al.*, 2008). That is why, an investigation of pathogenic flora that indicates a non respect of good hygiene practice especially fecal contamination is required.

It can also be seen from Table 1 that, the load of total aerobic flora decreases significantly ($p < 0.05$) with time of sampling (J_1 and J_4) whatever the manufacturing process used. The decrease observed in the total aerobic flora with time could be explained by the reduction of the pH of the medium leading to inhibition of some microorganisms.

Total and fecal coliforms

The level of contamination of the different samples of *pendidam* by coliforms has been investigated. It arises from Table 1 that the load of total coliforms of the *pendidam* sample ranged from $13.00 \pm 0.01 \times 10^2$ CFU/mL (sample PM_1 at J_1) to $4.01 \pm 0.07 \times 10^4$ CFU/mL (sample PM_2 at J_4). These results show that *pendidam* produced and sold in Ngaoundere represents a risk for consumers and also for public health because the load of coliforms in all samples were higher than those recommended by the standard concerning fermented milk (<10 CFU/mL). Similar observations have been reported by Tiku *et al.* (1999) who found that total coliforms count was 1×10^5 CFU/mL on *pendidam* sampling at Adamaoua region and western highlands of Cameroon. Dongmo *et al.* (2008) found total coliforms count of $5.16 \pm 0.14 \times 10^2$ CFU/mL on *pendidam* sampling at Ngaoundere. Katinan *et al.* (2012) found that the total coliforms count of fermented milk manufactured and sold in the town of Yamoussoukro varied from $2.80 \pm 4.86 \times 10^4$ CFU/mL to $7.12 \pm 6.32 \times 10^4$ CFU/mL. The presence of coliforms at high level than those recommended by the standard could be explained by the fact that the methods of production of the various traditional fermented foods are usually primitive, compared to modern ways of food processing (Dirar, 1997). The major risk enhancing factors could be the use of

contaminated raw materials, the lack of pasteurization in some cases, the use of non controlled or natural fermentations, and inadequate storage and maturation conditions.

Concerning fecal coliforms, they were present in all samples of *pendidam* with a load ranging from 1.25 ± 0.05 x 10^3 CFU/mL (sample PM_1 at J_1) to 3.35 ± 0.11 x 10^4 CFU/mL (sample PM_2 at J_4). Their loads in all samples of *pendidam* were higher than those recommended by the standard. The presence of fecal coliforms in all samples could be explained by the non-respect of good hygiene practices during the manufacturing process because fecal coliforms are microorganisms whose presence in foodstuffs indicates fecal origin of contamination (Briand, 2007; Ghafir, 2008). It can also been noticed from Table 1 that total coliforms count of *pendidam* samples increased significantly ($p < 0.05$) with time and manufacturing process. Similar observations have been obtained with the fecal coliforms count of these samples. This observation could be explained by the fact that coliforms are able to ferment milk and some of them tolerate acidic pH, and by the antimicrobial effect of pasteurization because the *pendidam* manufactured with process A presented a low microbial load compare to those manufactured with process B.

Yeast and moulds

Concerning investigation on yeasts and moulds, it arises from Table 1 that, yeasts are present in all samples of *pendidam* regardless of the storage time and manufacturing process, but moulds were present only in sample PM_2 collected at the day of manufacture (J_1) with a load of 20 ± 1 CFU/mL. Similarly, Hosono *et al.* (1989) found an appreciable number of yeasts (1.1 x 10^7 CFU/mL) in samples of *Dadih*, an Indonesian fermented milk which is made by pouring buffalo milk into fresh bamboo tubes and capping them with banana leaves. Jiwoua and Millière (1990) in their studies on *pendidam* sampling at Ngaoundere, found a microbial load of yeast and moulds ranging from 1 x 10^3 to 1 x 10^9 UFC/ mL. Loretan (1999) found a yeast count of 4.1 x 10^6 CFU/ mL in indigenous traditional South African fermented milks. The presence of yeasts in all samples of *pendidam* at different load may be influenced by the age of the product as well as the containers and processing methods used (Beukes *et al.*, 2001) or may be due to the acidophilic character of yeast and moulds and their lower sensitivity to the antagonistic activity of lactic acid bacteria (Benkerroum *et al.*, 1984). By the way, the load of yeast increase significantly ($p< 0.05$) between J_1 and J_4 irrespective of the manufacturing process, and remained above the value recommended by the standards ($< 10^2$ CFU/mL). The increasing level of yeasts with time could be explained by the acidophilic character of yeast and moulds and their lower sensitivity to the antagonistic activity of lactic acid bacteria as previously reported.

Fecal *Streptococci, Salmonella, Staphylococcus aureus* and sulphito-reducing *Clostridium*

Other microorganisms investigated in this study were fecal *Streptococci, Salmonella, Staphylococcus aureus* and sulphito-reducing *Clostridium*. All of them were absent in the samples of *pendidam* analyzed except *S. aureus* which were present in some of the samples probably due to the manufacturing process. The absence of these pathogenic germs in the samples of *pendidam* could be explained by the presence of lactic acid bacteria which contribute to the improvement of the shelf-life and safety of the food by producing

several antibacterial compounds including lactic and other organic acids, ethanol, hydrogen peroxide, carbon dioxide, diacetyl, bacteriocins and biosurfactants (Velraeds *et al.*, 1996; Caplice and Fitzgerald, 1999; Rodrigues *et al.*, 2006).

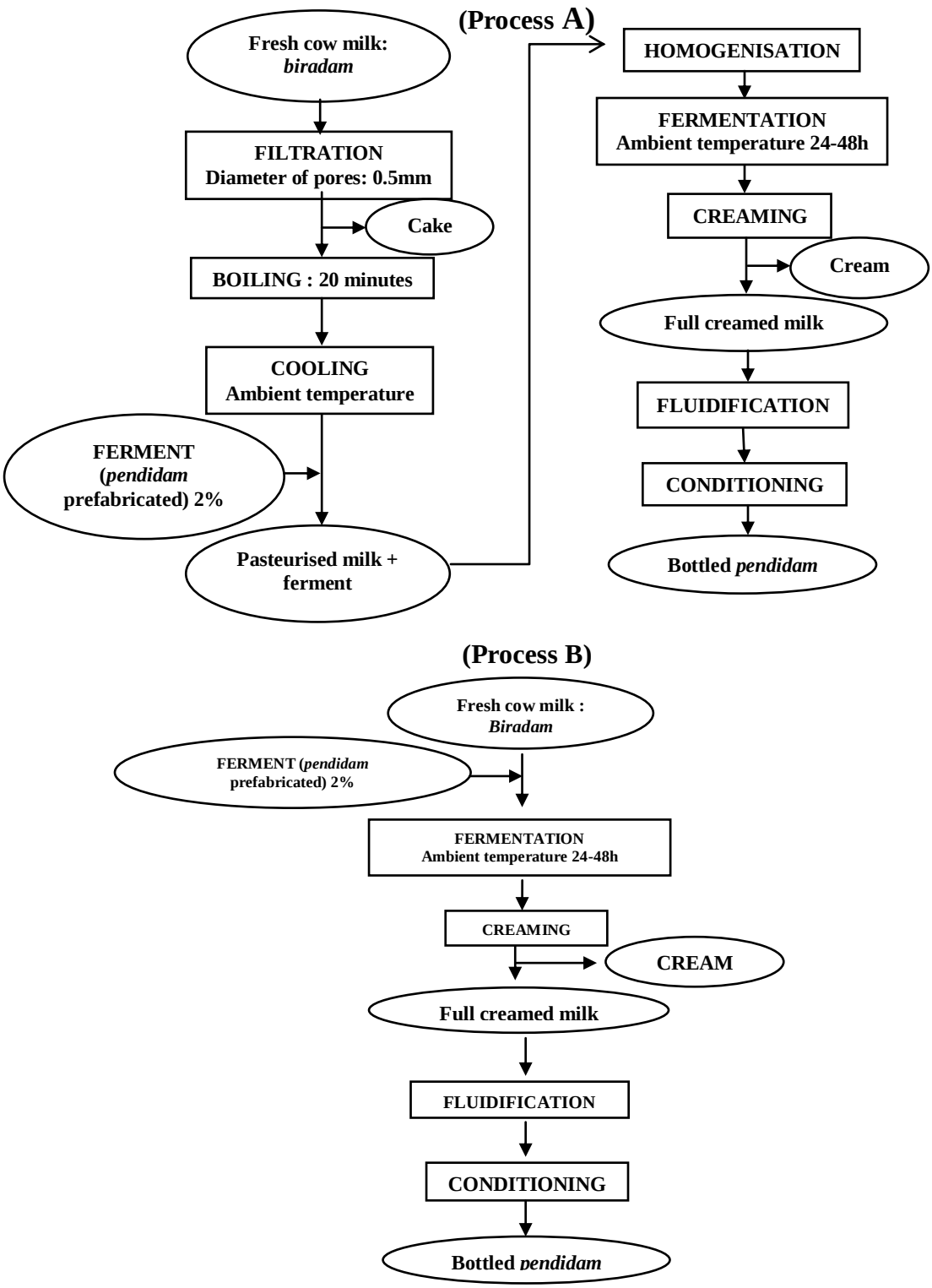
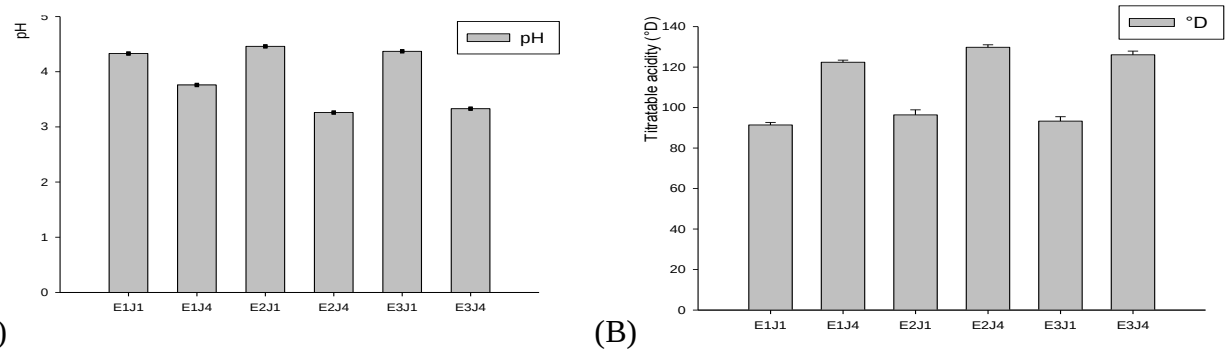


Figure.1 Process A and process B of manufacturing *pendidam*



(A) (B)

Figure.2 pH (A) and titratable acidity (B) of *pendidam* samples (E_1 = PM_1 = sample of Dang-market, E_2 = PM_2 = sample of the small market of Ngaoundere, E_3 = PM_3 = sample of Wakwa, J_1 = sample collected at the day of manufacture, J_4 = sample collected four days after manufacture)

Table.1 Rate of contamination of pendidam samples (cfu/mL)

Micro-organisms	PM_1 (process A)		PM_2 (process B)		PM_3 (process A)		Standard	Evolution
	J_1	J_4	J_1	J_4	J_1	J_4		
Total flora	$9.8 \pm 0.14 \times 10^{6c}$	$1.60 \pm 0.05 \times 10^{5a}$	$2.20 \pm 0.07 \times 10^{7d}$	$2.94 \pm 0.05 \times 10^{5a}$	$8.45 \pm 0.04 \times 10^{6b}$	$8.40 \pm 0.14 \times 10^{4a}$	$<10^6$	↓
Total coliforms	$5.00 \pm 1.41 \times 10^{3a}$	$13.00 \pm 0.01 \times 10^{2b}$	$1.13 \pm 0.02 \times 10^{4d}$	$4.01 \pm 0.07 \times 10^{4e}$	$7.65 \pm 0.49 \times 10^{3a}$	$9.2 \pm 0.49 \times 10^{3e}$	<10	↑
Fecal streptococci	-	-	-	-	-	-	<10	-
Yeasts	$2.45 \pm 0.21 \times 10^{4a}$	$9.45 \pm 0.35 \times 10^{4c}$	$1.97 \pm 0.19 \times 10^{5d}$	$3.50 \pm 0.14 \times 10^{5e}$	$2.15 \pm 0.77 \times 10^{4a}$	$4.15 \pm 0.49 \times 10^{4b}$	$<10^2$	↑
Moulds	-	-	20 ± 1^{a}	-	-	-	<10	?
Staphylococcus aureus	-	-	13.00 ± 0.50^{c}	3.00 ± 0.01^{ab}	7.00 ± 0.11^{b}	2.00 ± 0.01^{a}	<10	↓
Fecal coliforms	$1.25 \pm 0.05 \times 10^{3a}$	$2.63 \pm 0.12 \times 10^{3b}$	$2.70 \pm 0.06 \times 10^{3b}$	$3.35 \pm 0.11 \times 10^{4c}$	$1.4 \pm 0.49 \times 10^{3a}$	$2.48 \pm 0.07 \times 10^{3b}$	<1	↑
***Salmonella* spp.**	-	-	-	-	-	-	Absent	-
Sulphito-reducing *Clostridium*	-	-	-	-	-	-	Absent	-

Means ± SD ; Means within the same column lacking same superscript numbers and means within the same line lacking same superscript letters are significantly different at $p<0.05$. PM_1 = sample of the market of Dang; PM_2 = sample of the small market of Ngaoundere town; PM_3 = sample of Wakwa; J_1 = sample collected at the day of manufacture; J_4 = sample collected four days after manufacture.

In conclusions, at Ngaoundere town and the neighbouring markets, two main processes are used to manufacture *pendidam*: the first which included pasteurization as a unitary operation of the process and the second in which this operation is absent. The microbiological analysis of *pendidam* collected in different localities of Ngaoundere shows that, total and fecal coliforms, *Staphylococcus aureus*, yeasts and moulds, fecal *Streptococci* are present in all samples. *Pendidam* manufacturing with the process which included pasteurization have presented the lower microbial load. However, the microbial loads of all pendidam samples were higher than those recommended by standards for fermented milks. The non-respect of good hygiene practice and good manufacturing practice are the main causes of this poor quality of *pendidam*. Coliforms and yeasts which loads increase with time could be considered as micro-organisms responsible for the spoilage of *pendidam*.

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