

Preparation of the starter Trial production of cheese (Jben) and Klila at laboratory scale

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Abstract

Samples of bulk milk collection center in the municipality of Mograne have been selected to prepare the mesophilic and thermophilic lactic yeast.

Two types of yeast (mesophilic and thermophilic) have been selected for their acidifying power, the speed of coagulation and aromatic aspect to produce two types of cheese, fresh cheese (Jben) and Klila. Physicochemical and microbiological tests were conducted for four by-products Jben and Klila prepared by the traditional method and Jben and Klila at laboratory scale.

The analysis included five samples of each coproduit. The pH of the samples vary between 3.62 and 3,92 while the acidity is between 66 and 95 °D. Clotting time of milk is too fast for thermophilic starters (3,50 - 4 hours cons to 11, 50 -10, 50 for mesophilic starters). The microbiological analysis have highlighted a complete absence of coliforms, and staphylococci, especially for controlled products. The finished product obtained had a considerable consistency and texture besides a very satisfactory organoleptic and hygienic quality according to the physicochemical and microbiological analysis carried.

Keywords: starter, thermophilic, mesophilic, Jben, Klila

1. Introduction

Milk and dairy products are staple foods for humans. The bovine species constituting the principal source of milk production for human consumption. Cheese is a dairy product most valued by humans for a long time. However, there are more than 1000 varieties produced worldwide (Hayaloglu *et al*, 2002).

The name "cheese" is reserved to fermented product or not obtained by coagulating milk, cream, skim milk, or a mixture thereof, followed by draining. The cheese is made either by the traditional method in the rural environment and traditional dairies (Mahlabas) or by the method semi-industrial or industrial methods which remains limited (Mennane, 2008). The Jben is a dairy product known and consumed in Morocco for a long time in both rural and urban areas (El Marrachi and Hammama, 1996).

The national production of cheese from industrial units is 3391 tones in 1982 and 4.546 tones in 1983. Productions of small farm units, although not included in the calculation of the total production, nevertheless represent an important part of the national consumption of cheese. (Hammama, 1989a)

In Morocco, Klila and Jben (cottage cheese) are very popular local products (traditional cheese) (Mennane *et al*, 2007) and very appreciated in national scale according to a standard protocol which includes rennet (enzyme extracted from the stomach of veal or lamb's stomach or industrially produced from molds used to curdle milk. (Larousse) for Jben and leben (spontaneously fermented product and devoid of butter after churning) to prepare the Klila. Studies and references on cheeses made traditionally in Morocco (Klila, Jben) are very rare and shy with the exception of the doctoral thesis of Mennane 2008, which tried to improve these products.

The objective of this work is to try to make the Jben and Klila with the traditional procedure and the laboratory scale, with a physicochemical and microbiological comparative study to highlight the nutritional and hygienic quality of these products.

2. Materials and Methods

2.1 Preparation of lactic starters

- Preparation of thermophilic natural leaven.

Five sterile vials are filled with 250ml of raw milk. They are incubated for 24 hours at 45 °C. Floras that are developed are considered the population of the first generation G1. The second generation (G2) is obtained by

incubation in the same conditions as above, each of the flasks containing 90 ml of sterile raw milk and 10 ml of the G1. Three other generations were prepared: the third, fourth and fifth. They were obtained by using the same protocol adopted for the G2. The fifth generation was used for the isolation of lactic bacteria.

- *Preparation of mesophilic leaven.*

The same procedure as above was followed to prepare the mesophilic yeast. The only change is at the temperature of incubation. It was set at 30 °C. Similarly, the fifth generation was used for the isolation of lactic bacteria.

2.2 Measure of pH

PH measurement is done by a pH meter Orion Research type with a combined electrode and previously calibrated with buffer solutions at pH = 4 and pH = 7.

2.3 Measurement of the acidity

The acidity is calculated by titrating 10 ml of the milk sample with a solution of sodium hydroxide (NaOH) of normality N /9 in the presence of a colored indicator (phenolphthalein to 1%). The acidity is expressed in degrees Donric (°D) ($1^{\circ}\text{D} = 0.1\text{g lactic acid/liter}$). To 10 ml of sample to be analyzed, adds a drop of 1% phenolphthalein. Soda N/9 contained in the Mohr burette with stopcock is versed in drop into the beaker containing milk phenolphthalein. The fall of the drop is followed by homogenization. In turn to whitish color, the burette stopcock is turned off to record the volume of versed soda.

2.4 The starter selection

The choice of a starter is based on its acidifying power, speed of coagulation, its aromatic power and the finished product texture. These characteristics will serve us to select thermophilic leaven and mesophilic leaven.

2.5 Fabrication of Moroccan-controlled Klila

- Preparation of milk

6 liters of raw milk of cows were collected and transported to the laboratory. The milk is made at the rate of a liter and a half in Meyer flasks of 2 liters of usable capacity. It is pasteurized in a bain-marie at a temperature of 65 °C for 30 min.

- Seeding

After pasteurization, the milk is cooled to 45 °C, and then inoculated with a thermophilic starter culture at 10 %. The incubation was carried out for 4 hours (time necessary to obtain a correct coagulation)

Meanwhile a second experiment is triggered. It is the production of cheese which technical progress is carried at a temperature of 30 °C. The activity consists in seeding pasteurized milk by a mesophilic starter. The inoculum was added at a rate of 10%. The incubation period in this case is carried out in a water bath at 30 °C for 10 hours.

- Thermic treatment (72 °C/15min)

This step accelerates the coagulation process, appears the hardening phase and promotes the edification of curds granulation with diffusion of fat to the whey.

- Molding and draining.

Molding is performed by depositing the coagulum in perforated plastic molds. The assembly is covered with a cloth to prevent attack by insects. The perforations of the plastic container facilitate the exit of the liquid phase linked to the curd. The nature of the perforations and the time of rest are our success factors of the draining operation. This last is a fundamental step. It may take a few hours to a few days. And it depends on the required taste and consistency.

2.6 Preparation of Jben

- Preparation of milk

6 liters of raw milk of cows were collected and transported to the laboratory. The milk is made at a rate of a liter and a half in Meyer flasks of 2 liters of usable capacity. It is pasteurized in a water bath at a temperature of 65 °C for 30 min.

- Seeding

After pasteurization, the milk is cooled to 45 °C, then inoculated with a thermophilic starter culture at 10%. Incubation is carried out for one hour (time necessary to obtain a correct coagulation). We can add at this stage the rennet used to curdle milk, and then we reincubate during an hour still to 45 °C.

- Molding and draining.

Molding is performed in the same manner described for klila. To recall, at the time of fabrication of Jben, and particularly between draining and ripening, we added salt. It is a complement of draining and an important factor in controlling the maturing with an action on the water activity (Riahi, 2006).

2.7 Analytical evaluation of the processing products

After processing of milk, the two transformation products (Jben controlled, Klila controlled) were subjected to an analytical assessment. The objective is to define the difference in nutritional quality between our finished product and the product sold in traditional dairies in Kenitra city. We have made their microbiological and physicochemical analyzes (pH and acidity).

- *Physicochemical analysis*

The materials and methods for the measure of pH and acidity are those described in page 2.

- *Microbiological analysis*

Microbiological analyzes are carried out for the sourdough used, Klila controlled, Jben controlled in order to look for:

- The indicators of the hygienic quality of milk which are:

- The enumeration of total aerobic mesophilic flora (FMAT) is enumerated on PCA agar (Plate Count Agar) incubated for 24 h at 30 °C.

- The search for total and fecal coliforms: Coliforms are sought on desoxycolate citrate agar (DCL) incubated for 24 hours at 37 °C for total coliforms and at 44 °C for fecal coliforms.

- Germs of industrial interest:

- A count of lactic acid bacteria responsible for the fermentation and acidification of milk, they are counted on the MRS agar (Man Rogosa Sharpe, Difco, Detroit, USA) and incubated for 48 hours at 30 °C.

- The yeasts and molds were counted on potato dextrose agar (PDA) and incubated for 48 hours at 30 °C.

- Research of harmful bacteria (pathogens):

- Fecal streptococci were counted on sodium azide after incubation for 48 hours at 37°C.

- Staphylococci are counted on Chapman medium (or Mannitol Salt Agar medium) containing a high concentration of NaCl (75%) tolerated only by staphylococci, incubation is at 37 °C for 24 to 48 hours.

- For Salmonella, there is provided a pre-enrichment on selenite-cysteine medium for 12 hours at 37 °C, followed by an enrichment on bouillon of tetrathionate for 24 hours at 37 °C, then the enumeration and isolation were carried out on SS medium (Salmonella-Shigella) after 24 hours of incubation at 37 °C.

- The sulphitoreductor-clostridia are counted in the culture medium reinforced Clostridium Agar in tubes to promote anaerobic conditions, with thermic treatment for 10 min at 80 °C to activate the spores of clostridia: they can persist in a latent form in milk, germinate as soon as conditions are favorable and secrete toxic substances. The tubes are incubated for 48 h at 37 °C. Only black colonies are counted.

The microbiological analysis is performed in three steps: preparation of dilutions, seeding in the culture medium and enumeration of microorganisms.

2.8 Preparation of Jben (white cheese) controlled in laboratory

- Preparation of milk

4 liters of raw milk of cows were collected and transported to the laboratory.

• Pasteurization

The milk is placed in 2-liter Erlenmeyer flasks and subjected to pasteurization in a water bath at a temperature of 65 °C for 30 min.

• Seeding with leavens in the order of 10%.

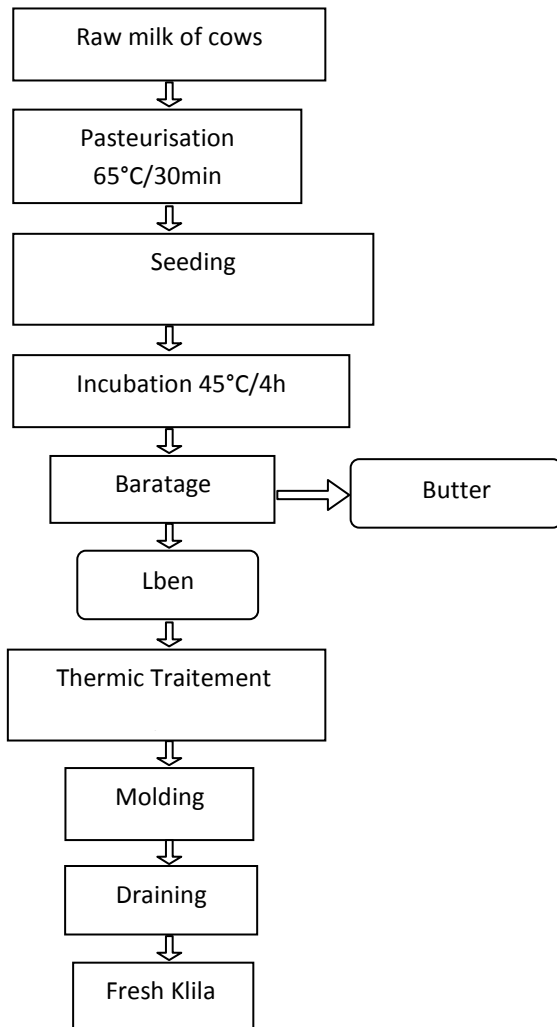
• After pasteurization the milk is cooled to 45 °C, and then inoculated with thermophilic starter (LVt1, LVt2, LVt3) at the rate of 10%. Incubation lasts an hour until coagulation and decrease in pH. We can add at this stage the rennet used to curdle milk, and then we reincubate during an hour still to 45 °C.

• Molding and Drainage

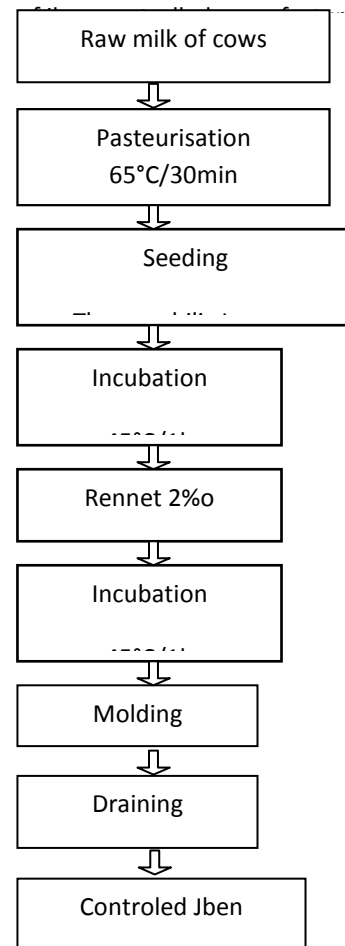
Molding is performed after cooling the coagulant in perforated plastic molds covered by a cloth fine filter to recover the maximum amount of curd to allow for proper draining.

Draining can last from a few hours up to a few days depending on the desired flavor and consistency.

The following diagram illustrates the protocol of Klila controlled manufacturing



The diagram below illustrates the protocol



3. Results and Discussion

3.1 Physicochemical analysis

The selected yeasts present a pH ranging between 3.60 and 3.92 with an average of 3.76 for thermophilic starter and an average of 3.55 for mesophilic. These values are lower than those found by Mennane, (2008) which are respectively 4.12 and 3.66.

The acidity registers an average of 82 °D for thermophilic starter and 87 °D for the mesophilic, while for Mennane, averages are of the order of 94.80 and 120 °D.

The values of the coagulation time are consistent with those of Mennane, they vary between 3.50 and 4 hours for thermophilic starter cultures (Table I).

The leaven T.lev2 was chosen for the preparation of our products, in view of its high acidity, its short duration of coagulation and its appreciable flavoring smell.

3.2 Fabricated dairy products

- *Physicochemical Analyses*

Our prepared products have recorded pH averages for the five samples of each type ranging from 3.89 to 4.26 (Table II). These values are between the values found by Mennane, (2008) and which have a maximum of 4.70 and a minimum of 3.80. Bayi, 1990 and Sriti, 1996 found values which are respectively 4.02 and 4.54.

The acidity averages generate values between 76 and 97 °D located in the interval averages announced by Hammama 1989b (99 °D) and Kbibou, 1987 (111,60 °D).

3.3 Microbiological analysis of prepared products

Microbiological analyzes have shown a load of FMAT about 9.10^4 cfu /g for controlled Klila and $1,2.10^6$ cfu /g for the traditional Klila while for the controlled Jben the recorded value is 3.10^4 cfu / g against $2,8.10^6$ cfu /g for traditional Jben. These values are similar to those found by Mennane, 2008 and ranging from $1,43.10^5$ cfu /g to $1,01.10^6$ cfu /g and remained lower than those found by other authors as Moroccan Hammama 1989b Mahi and al, 1995 Aboulala and al, 1994 Zahar and al, 1997 and El Marrakchi, 1988 for fresh cheese of Morocco.

We also noticed the absence of pathogenic flora especially for controlled products. This is due to controlled heat treatment and hygiene practices during the production.

The load of lactic acid bacteria is $2,5.10^4$ cfu /g for the controlled klila and $1,8.10^4$ cfu /g for the traditional klila. The controlled Jben marks a value of $9,7.10^4$ cfu/g and $5,9.10^3$ cfu /g in for the traditional jben (tab III). The values reported by Mennane, 2008 are between 10^3 and $1, 2.10^4$ cfu/g.

4. Conclusion

The dairy sector in Morocco, defined through its four main links: production, collection, processing - marketing and consumption, experienced a dynamic evident during the last three decades.

The results presented in this study show the importance of the use of raw milk, with a good microbiological quality in the manufacture of cheese (Hammama 1989b).

Milk is a very complex and unstable mixture. Its production must be tightly controlled because of the possible risks which may present to human health (Labioui and al, 2009). The search for microorganisms indicators of fecal contamination can judge the hygienic condition of the product. Even at low levels, they are evidence of hygienic conditions deteriorated during milking or during transport. Must nevertheless establish a quality policy with the popularization of good farming practices, and insist on clean animals, their immediate environment and the safety of trafficking.

For this, it is essential to implement good hygiene practices of milking and rapid and adequate refrigeration of milk after its production just at its use. We must also remember that vigilance and caution during handling the preparation of fresh cheese avoid the contamination of milk either by manipulators or by dairy equipment used.

The adoption of modern and suitable manufacturing processes for the preparation of Jben and Klila avoids the contamination by various pollution germs and get a safe product complies with international microbiologies.

Also the use of some selected yeasts leads to obtaining superior sensory quality products. (El Marrachi and Hmama, 1996).

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Photo 1. Different lactic leavens prepared



Photo 2. Different products manufactured in the laboratory

Table I. Averages of pH, acidity and duration of coagulation of prepared yeasts

| Leaven of the 5th generation | pH | Acidité D° | Duration of coagulation in heure |
|------------------------------|-------|------------|----------------------------------|
| T.Lev1 | 03,60 | 66 | 03.50 |
| T.Lev2 | 03.92 | 98 | 03.50 |
| T.Lev3 | 03,67 | 69 | 04 |
| Average | 3.76 | 82 | 03,75 |
| M.Lev1 | 03,58 | 85 | 10.50 |
| M.Lev2 | 03,52 | 89 | 11,50 |
| Average | 03,55 | 87 | 11 |

T.Lev : thermophilic leaven

M. lev : mesophilic leaven

Table II. pH averages and acidity of prepared cheeses

| | Average of 5 samples | | | |
|-------|---------------------------------|----------------------|-----------------------|----------------------|
| | Klila1 (T.lev2) (controlled) | Klila2 (traditional) | Jben1 (controlled) | Jben 2 (traditional) |
| PH | 04,09 | 03,89 | 04,18 | 04,26 |
| AC D° | 97 | 88 | 83 | 76 |

Table III. Microbiological analyzes microbiologiques of prepared products (Klila, Jben)

| samples Average of 5 tests in cfu /g | FMAT | Coliformes | | Staphylococci | Lactic Bactia | Yests |
|--|------------|------------|-------|---------------|------------------|------------|
| | | total | fecal | | | |
| Klila1 | $0,9.10^5$ | 0 | 0 | 0 | $2,5.10^4$ | $1,9.10^4$ |
| Klila 2 | $1,2.10^6$ | 210 | 112 | 0 | $1,8.10^4$ | $3,1.10^4$ |
| Jben 1 | $0,3.10^5$ | 0 | 0 | 0 | $9,7.10^4$ | $0,6.10^4$ |
| Jben 2 | $2,8.10^6$ | 0 | 0 | 0 | $5,9.10^3$ | $2,8.10^4$ |