

MICROBIOLOGICAL AND PHYSICO-CHEMICAL QUALITY OF RAW MILK OF BENI MELLAL-KHENIFRA

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ABSTRACT

Background. Milk is a nutritious food for humans, but it is also an ideal growth medium for pathogenic bacteria such as *Salmonella*, *Listeria monocytogenes* or *Campylobacter* spp, which can cause food poisoning.

Objective. For this reason, we evaluated the microbiological, physicochemical and hygienic quality of raw milk samples in 9 milk collection centres and cooperatives in the Beni-Mellal-Khenifra region before and after the implementation of the quality management system, hazard analysis and critical control points (HACCP).

Materials and Method. The microbiological and physico-chemical quality of 184 raw milk samples in 9 milk collection centres and cooperatives in the Beni-Mellal-Khenifra region was evaluated before and after the implementation of the quality management, hazard analysis and critical control points (HACCP) system.

Results. The physico-chemical results obtained after the implementation of HACCP revealed that 78% of the samples analysed were below the maximum limits authorised at international level. A pH between 6.6 ± 0.11 and 6.8 ± 0.02 , an acidity of 15°D, the milk is stable according to the Ramsdell test which is negative at 0.9. The temperature remains within the acceptance range with an average of $6.62 \pm 0.45^\circ\text{C}$ to $7.7 \pm 0.36^\circ\text{C}$ and the fat content is between 33 and 45g/ml. We also noted an improvement in the microbiological quality of milk in 81% of collection centres and cooperatives. The level of contamination by total coliforms is from 2.5×10^4 to 11.5×10^4 CFU/ml lower than the Moroccan standard (NM 08.0.100) of 1.5×10^4 CFU/ml to 20.3×10^4 CFU/ml, the total aerobic mesophilic flora and the psychrotrophs have an average of 2.5×10^5 germs/ml to 82×10^5 germs/ml lower than before improvement (12.5×10^5 germs/ml to 192×10^5 germs/ml).

Conclusion. We can conclude that the work done leads to an improvement and satisfaction of the quality of raw milk compared to the standard after the implementation of HACCP.

Key words: raw cow milk, milk hygiene, physicochemical quality, HACCP, microbiological load

INTRODUCTION

In Morocco the dairy sector is developing despite the presence of several difficulties [24]. Milk and dairy products, are the most consumed food products in many countries, and provide a favorable environment for the growth of many microorganisms due to their richness in macro- and micronutrients [35]. In fact, raw milk contains essential nutrients in a balanced way such as lactose, fat, protein, minerals, and vitamins more than most other foods [20]. In recent years, the consumption of raw milk has become increasingly popular due to its potential benefits, such as its high nutritional content and also its richness in beneficial bacteria, as well as the prevention of lactose intolerance [18]. In developing countries, the dairy sector accounts for up to 40% of agricultural gross domestic product (GDP) and 4% of national GDP [36].

Raw milk can be contaminated either endogenously by microorganisms excreted with the milk, or exogenously by microorganisms introduced during or after milking. In the first case, microorganisms are excreted with the milk during or after milking either from the udder skin or from the environment (feces, dust, equipment, humans, etc.) [39]. Also milk is more widely influenced by environmental and genetic factors like any other biological fluid [33].

Raw milk quality is important for potential technological processing into by-products [20]. For this reason many studies have been conducted to reduce the risk of microbial contamination and increase the chemical nutritional quality of dairy products [34, 35]. However, raw milk can contain pathogens and their toxins, which can cause serious foodborne illness [19]. Consumers cannot judge the safety of milk based on its organoleptic qualities (taste, appearance, and

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odor), but rather they must be able to source raw milk from farmers who have adopted a high level of good husbandry and manufacturing practices to reduce the potential risks associated with consuming raw milk [9].

This study aims to implement actions that will allow the reduction of the microbial load of raw milk in cooperatives and collection centers through the application of good hygiene practices at the enterprise level. For this reason, we evaluated the microbiological, physicochemical and hygienic quality of raw milk samples in 9 milk collection centres and cooperatives in the Beni-Mellal-Khenifra region before and after the implementation of the quality management system, hazard analysis and critical control points (HACCP).

MATERIALS AND METHODS

Study area

The dairy industry is contracted with 107 cooperatives, collection centers and farms including 36 in Beni-Mellal-Khenifra, 23 in the Chaouia region, 11 in Tifelet and 37 in the region of Doukkala and 3 farms (Settat, Berrechid). This work has concerned 9 cooperatives and centers in the region Beni mellal-Khenifra (Figure1).

Sampling

184 raw milk samples were collected aseptically in the presence of an air sterilizer and sampling space for physicochemical and microbiological analysis. A ladle is plunged into the tank through its upper opening for homogenization. Then a sample is taken and

placed in a sterile bottle with a screw cap. Sampling was conducted once a week from February through December 2021. For each sample, a 1 ml sample of raw milk to be analyzed was added to 9 ml of sterile peptone water. A stock dilution is obtained from which decimal dilutions are made.

Survey of collection centers and cooperatives

In order to evaluate the level of compliance with good hygiene practice at the level of the cooperatives and collection centers studied and to define the microbial load of raw milk, we have developed a questionnaire to assess the situation and to develop an action plan for the improvement of the studied sites. The evaluation of investigation information is structured as follows:

1. *Information on the collection center or cooperative:* contains the status, number of members and people working there. The examinations and tests used at the reception of milk.
2. *Information on the respondent and the owner:* this section provides information on the respondent's level of education, experience in animal husbandry, and position or responsibility other than president of the cooperative or center.
3. *Knowledge of good hygiene practices and their application in the cooperative or center:* this part is characterized by the wealth of data on the amount of milk received in liters per day and the direct marketing of this milk. It contains mainly information on body and clothing cleanliness, construction, equipment and instruments in contact

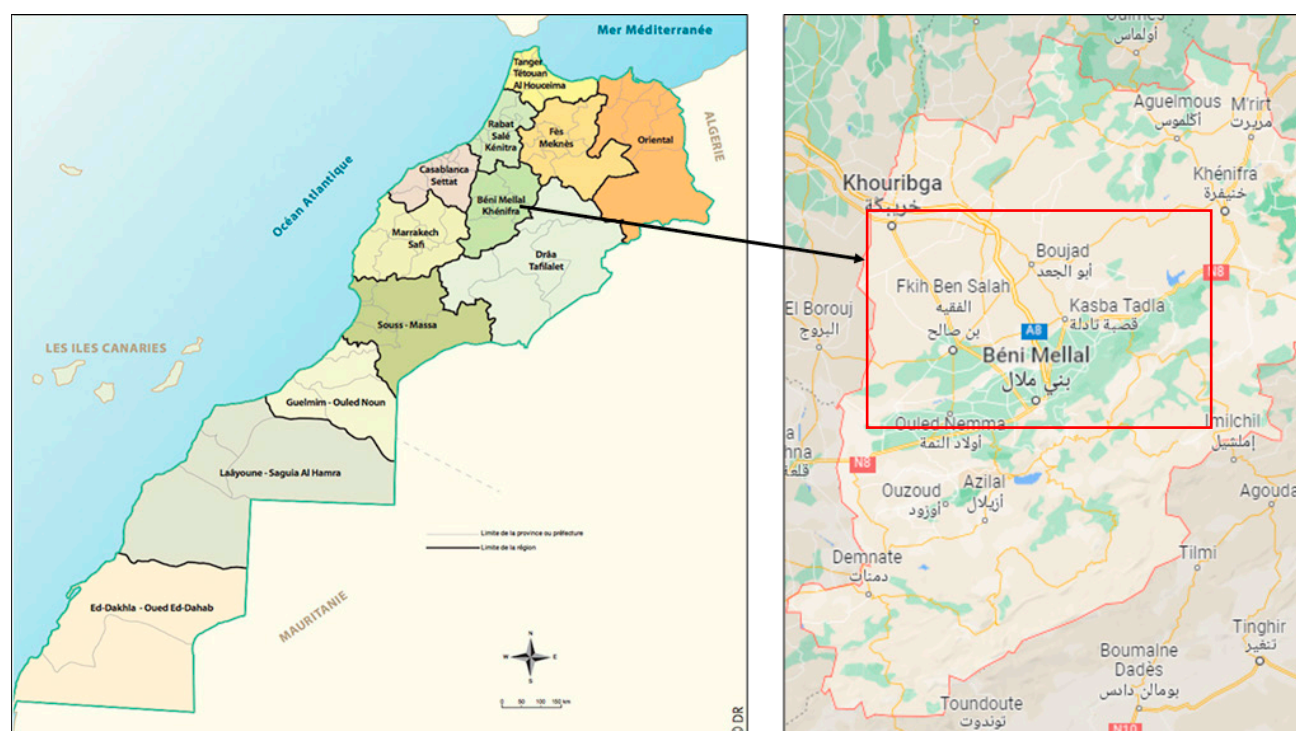


Figure 1: Location of collection centers and dairy cooperatives in the Beni Mellal-Khenifra region

with milk, the availability of control instruments and also some information on the farmer.

Data management

After completing our surveys, two databases Excel 2013 were created, one for the survey sheets for the breeders and another for the questionnaires for the breeders who own the collection centers.

Physicochemical analysis

All the physico-chemical analysis were carried out according to the methods and procedures established by the bedding company in the company's laboratory:

Temperature measurement: The temperature is read directly on the scale of the digital thermometer (DT150 Summit). It is expressed in °C.

Determination of titratable acidity: The titration of acidity is done by sodium hydroxide in the presence of phenolphthalein as indicator [25]. The results are expressed as follows:

$$AC = V \cdot 10 \text{ (expressed in Dornic).}$$

Where: AC: Titratable acidity, V: volume of the solution (NaOH) N/9

pH measurement: Measured with a pH meter (Microprocessor pH Meter, pH 211, HANNA Instruments). The latter is calibrated with buffer solutions at pH= 7, at a temperature of 20°C.

Alcohol test: The alcohol test is used to diagnose the stability of milk. The results are expressed by the presence of flocculation after addition of alcohol 68%, 76% or 79%. If the test is positive (+): the milk is unfit for heat treatment and in the absence of flocculation the test will be negative (-).

Fat: The determination of the fat is based on the dissolution of the fat to be measured by sulfuric acid (Sigma-Aldrich Germany). Under the influence of centrifugal force and the addition of a small amount of iso amyl alcohol, the fat separates into a clear layer whose graduations of the butyrometer reveal the rate.

$$MG \text{ (g/l)} = (B - A) \times 10$$

Where: A: reading of the lower level of the butyrometer; B: reading of the upper level of the butyrometer

Ramsdell test: It can assess the stability of milk to heat treatment according to its balance, namely the ratio Ca/P. Prepare a series of tubes containing increasing amounts of monopotassium phosphate solution: 0.9; 1; 1.1; 1.2; 1.3; 1.4 ml. The results are expressed by the presence of flocculation: if the test is positive (+), the milk is unfit for heat treatment and in the absence of flocculation the test will be negative (-).

Microbiological analysis

The microbiological analysis of raw milk, was performed by enumeration of *total aerobic mesophilic flora* (TAMF), *total coliforms* (TC), *fecal coliforms* (FC) and *psychrotrophic* bacteria. Sample preparation and decimal dilutions were performed according to Moroccan standard NM 08.0.100 [28].

Enumeration of total mesophilic aerobic bacteria (TAMF) and psychrotrophs

The enumeration of total germs remains the best method to estimate the index of safety and quality of milk. From decimal dilutions ranging from 10^{-1} to 10^{-6} (of peptone water and 1 ml of raw milk), aseptically transfer 1 ml into an empty petri dish prepared for this purpose and numbered. Complete with 12 to 15 ml of PCA agar (Fluka, Sigma-Aldrich, India) melted and cooled to $45 \pm 1^\circ\text{C}$. The plates will be incubated lid down at 30°C for 72 h, and the psychrotrophs will be incubated at 25°C for 48 h.

Enumeration of total and fecal coliforms (in solid medium)

From the decimal dilutions 10^{-1} to 10^{-4} , in an empty petri dish, complete with about 15 ml of VRBL agar (Scharleau, Spain) melted and cooled to $45 \pm 1^\circ\text{C}$. Then make circular movements to mix the agar with the inoculum [26]. The Petri dishes will then be incubated lid down for 24 to 48 h at:

- 37°C for the first series will be reserved for the research of total coliforms
- 44°C for the second series will be reserved for the research of fecal coliforms.

RESULTS

Survey results from different verified axis

The survey of milk production systems identified the parameters influencing the microbiological quality of raw milk. At the level of collection centers and cooperatives, the majority (78%) of the people working there have no education, one person has a primary education and another a secondary education. Also, the majority (67%) of the farmers have less than 10 years of experience in animal husbandry (Table 1).

According to Table 2, most of the collection centers and cooperatives visited have a refrigeration system and are connected to the electrical network. However, we noted a high rate of non-compliance, mainly in the areas of staff hygiene, cleaning and disinfection (Figure 2, 3).

From this score (Figure 2) we find that cooperatives number 3, 4, 5 and 7 have a low compliance rate compared to the others, especially at the level of personal hygiene, cleaning and disinfection, as well

Table 1. Individual information on respondents and collection center/cooperative presidents

Kind of respondent		No of responses
Level of education	None	7
	Koranic school	0
	Primary	1
	Secondary	1
	Superior	0
Years of experience	<10 years	6
	10-20 years	3
	>20 years	0

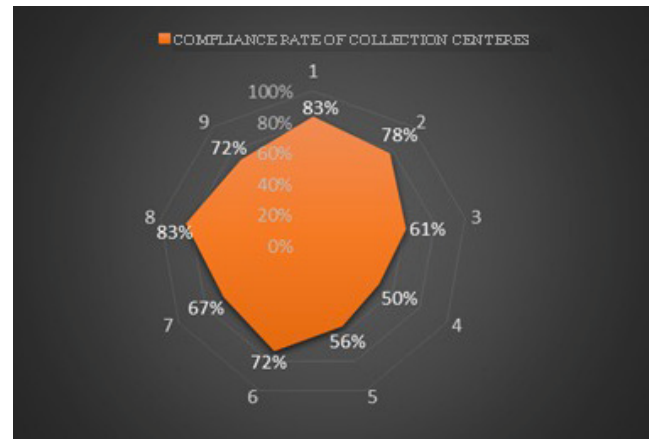


Figure 2. Compliance rates by center and by cooperative

Table 2. Action plan for improvement

Verified axis	Observations	Action/Improvement Plan	Statutes
Training	Some people in charge of these centers do not know that milk can transmit diseases	To propose continuous trainings for the persons in charge of the centers and cooperatives on: cleaning method; cold chain; waiting time; personal hygiene; sensitization of the persons in charge of the cooperatives and the farmers	The preparation and presentation of the training is done
Staff hygiene	Absence of gowns, gowns and gloves	Purchase of gowns, gowns and gloves	Waiting for budget validation
	Sinks (poor condition) are available in most centers and cooperatives, but lack soap and towels	Systematic maintenance of handwashing points, repair when needed, and availability of soap and towels	Follow-up, control and validation by the manager
Infrastructure and equipment	Walls and floors sometimes difficult to clean	Compliance with regulatory requirements	Follow-up, control and validation by the manager
	Lack of mosquito netting	Installation of anti-mosquito nets	Follow-up, control and validation by the manager
Cleaning and disinfection	Drinking water (from wells and castles)	Installation of water treatment systems (bleach; heat treatment)	Follow-up, control and validation by the manager
	Lack of hot water in cooperatives and centers with some exceptions	Installation of heaters	Follow-up, control and validation by the manager
	If no cleaning product is available; use a detergent (Tide) and bleach	Generalize disinfection and cleaning products on all centers and cooperatives	Validation by purchasing departments
Quality control	Control instruments are available but poorly cleaned	Awareness of good laboratory practices	Training
Cold chain	Refrigerator too close to the wall so no air intake and the temperature of the room increases	The temperature of the room must be controlled by placing thermometers in the centers and cooperatives	Follow-up, control and validation by the manager
	Lack of generator in some centers	Purchase of generators or installation of renewable energy systems (solar)	Validation by center managers

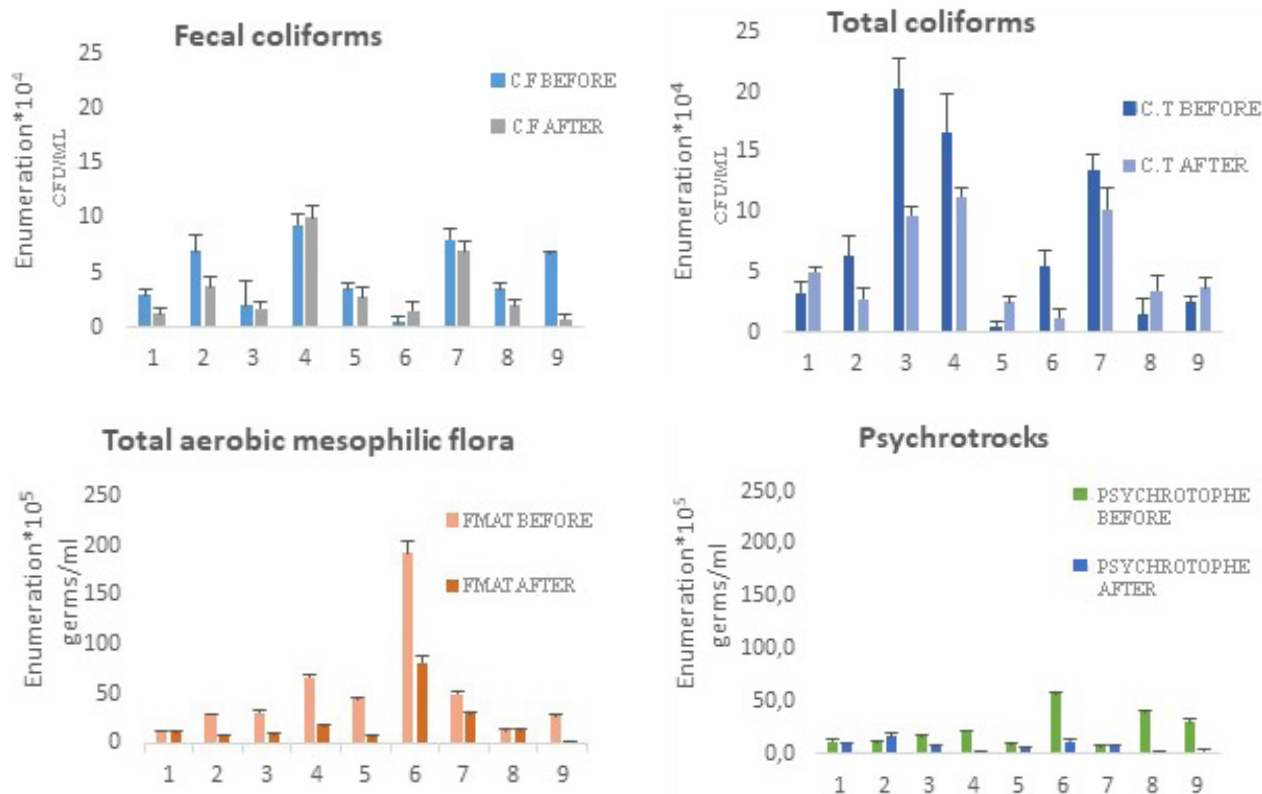


Figure 4. Microbiological analysis of raw milk from cooperatives and collection centers after improvement: Total mesophilic aerobic germs; fecal coliforms; total coliforms and psychrotrophs

increase in temperature in the rooms where there are refrigerators (absence of thermometers in the room and in the tanks). The time between milking and delivery to these cooperatives increases the risk of contamination.

Regarding the collection centers, the microbial load in total and fecal coliforms presents an average of 4×10^4 to 17×10^4 CFU/ml which remain within the internal standards of the company. However, significant alterations in the milk mix can reach critical values of $10^2 \times 10^4$. In order to follow the origin and source of this contamination, we find that the main cause is the absence of a control plan for cleaning the tanks and collection sites. The total aerobic mesophilic flora presents normal values except in one center where the load is close to 200×10^5 germs/ml, the psychrotrophs are high especially in only two centers. After our intervention at the level of hygiene improvement, we noted an improvement in the microbiological quality of milk in most collection centers and cooperatives. For total coliforms present an average of 2.5×10^4 CFU/ml to 11.5×10^4 CFU/ml with a low frequency compared to the starting 1.5×10^4 CFU/ml to 20.3×10^4 CFU/ml. The total aerobic mesophilic flora and the psychrotrophs present an average of 2.5×10^5 germs/ml to 82×10^5 germs/ml and a low frequency of contamination compared to the initial 12.5×10^5 germs/ml to 192×10^5 germs/ml. There is a decrease of the flora in most of the centers and cooperatives.

DISCUSSION

Dairy farms and collection centers

Managers and staff of dairy farms and collection centers have a low level of education regarding knowledge of the health risks of contaminated milk. Indeed, 78% have no education. It is therefore essential to make an effort in the supervision, training and awareness of these people. The place of collection of milk must meet the conditions set by the Moroccan standard N.M.08.4.050 on the guide to good hygiene practices for the production, collection and transport of raw milk, approved by joint order of the Ministry of Industry, Trade and Handicrafts and the Ministry of Agriculture, Rural Development and Maritime Fisheries No. 2301-98 [27]. The premises must be connected to the electrical network and connected to the Internet and/or must have the acquisition of a generator of sufficient power. Also, it must have a sufficient supply of drinking water, a hot water production system (water heater) for the cleaning and disinfection operations of the equipment and work utensils. It is also necessary to take into consideration the body and clothing cleanliness of the persons in charge and the personnel, to ensure an awareness and continuous trainings to have a change of the bad habits. In our study, more than 40% of the centers studied did not have a sink, a towel, soap at the entrance, a location on a concrete or tarred road, and a hot water

production system. This is in contradiction with the obligation of the Moroccan norm already mentioned. This can hinder the good hygiene of the raw milk received.

Physicochemical and microbiological analysis of raw milk from centers and cooperatives

In fact, according to the internal standard established by the company, the results obtained show that the temperature of 99% of the samples complies with the range of 4 to 10°C. Our raw milk samples comply with the regulation 853/2004 of the National Federation of Milk Producers: “primary production provides for hygiene during milking, collection and transport immediately after milking, the milk must be brought to a temperature not exceeding 8 °C when collected daily and 6 °C when not collected daily. “During transport, the cold chain must be maintained and the temperature of the milk must not exceed 10°C on arrival at the destination establishment”.

Milk acidity can be an indicator of milk quality at the time of delivery as it provides an indication of the amount of acid produced by bacteria or possible fraud [21] and also a decrease in pH results in lower water retention [11]. Fresh milk has a titratable acidity of 0.14 to 0.16% expressed as lactic acid [14]. Milk loses its keeping quality when a critical acidity reaches $0.200 \pm 0.01\%$ [6]. According to Mathieu, 1998 cow milk in early lactation has a titratable acidity of 19°D to 20°D. However, our values are between 14°D and 16°D, lower than those found by *Aggad* et al. [3], some samples had an acidity of 20°D. Except for the values for the mixture, which may exceed the national standards. This could be due to the different conditions that will change the state of the raw milk due to the poor hygiene of the tanks; their route; the time; and the collection of milk of poor quality (2 cooperatives) that can be mixed with that of good quality, which influences the total quality of the compartment. In addition, pH and acidity depend on the content of casein, mineral salts and ions, but also on the hygienic conditions during milking, the total microbial flora and its metabolic activity [23]. The alcohol test allows to determine the stability of raw milk, the *Ramsdell* test allows to confirm or to appreciate the stability of raw milk coming from different cooperatives. This criterion is very important because it allows the industrialist to fix the price for the farmer. The fat content of cow's milk varies from 35 to 45 g/L [5], and the FILAFNOR milk standard tolerates values between 34 and 36 g/L. *Agabriel* et al. [2] also showed that the genetic factor and the season also have an effect on the butterfat and protein content of milk. According to *Srairi* et al. [37], a diet rich in cellulose, which is the source of acetic acid, favors the butyrous rate [10].

Microbiological analysis of raw milk

The presence of total aerobic mesophilic flora (TAMF) is an important indicator of hygiene, and allows the evaluation of the bacterial quality of milk [31]. Indeed, before improvement we noted that all samples analyzed, have a total flora that is between 12×10^5 and 192×10^5 germs/ml. These results are consistent with the internal standards of the company (10^7 germs/ml) and close to that obtained by the study conducted in Burkina Faso by *Millogo* [32] which is 10^7 CFU/ml, but higher than those found by *El Marnissi* et al. [13] which is 4.5×10^5 CFU/ml. Several works [15, 37] as well as national regulations [22] agree that a load higher than 10^5 germs/ml means a significant contamination. These counts are also higher than the maximum loads tolerated by the two French and American regulations, which are 5×10^5 germs/ml and 3×10^5 germs/ml respectively [4]. Our results are in this range of 10^5 and 10^7 germs/ml which is similar to that found by *Ameur* et al. [7]. Thus our results are acceptable from the point of view of internal standards except at the level of two cooperatives where there is a lowering of the quality of raw milk received. This is due according to *Vietoris* et al. [40] to the use of unhygienic milking equipment, contamination of cow udders, inadequate cooling of milk, and sometimes by milking cows with mastitis.

The presence of fecal coliforms in raw milk indicates a source of environmental contamination. Their proliferation in raw milk reflects non-compliance with hygiene measures of the premises, tanks, and especially during milking [17]. The milk analyzed in this study before improvement had an average total coliform load of $4.7 \times 10^4 \pm 0.97$ CFU/ml for the centers and $11.8 \times 10^4 \pm 2.09$ CFU/ml for the cooperatives. These results are consistent with internal standards which are set from 4×10^4 to 400×10^4 CFU/ml. But higher than those reported in Morocco by *Afif* et al [1] which is 3.2×10^5 CFU/ml, and by *Ghazi et Niar* [16] in the region of Tiarret with an average of 1.7×10^6 CFU/ml. According to *Magnusson* et al [29], these high levels of coliforms are due to heavily soiled bedding. Thus other sources of contamination are also to be considered such as poor transport conditions. According to *Deeth* and *Fitz-Gerald*, [12] the presence of psychrotrophs is essentially a problem of temperature, the development of psychrotrophic flora at a level of 10^6 to 10^7 germs/ml in raw milk, generates the production of enzymes, especially lipases and proteases. Our results present an average load of 15.5×10^5 germs/ml, close to that obtained by *Triki* [38] at a dairy in Tunisia. The presence of these bacteria in raw milk is due to pollution that depends on the conditions of cleanliness of milking and equipment for collection, transfer and conservation of milk, the

quality of cleaning and rinsing water and the mode of feeding livestock.

Intervention for improvement

In order to improve the quality of raw milk, we proposed to the cooperatives and centers to apply the following interventions: To make continuous training for the persons in charge of the centers and cooperatives, concerning the method of cleaning, the cold chain, the waiting time, the hygiene of the personnel and the systematic maintenance by the person in charge of the zone of the points of washing of the hands (repair in case of need and availability of soap and towel). Thus, the installation of anti-mosquito nets in some centers and in a cooperative, the generalization of cleaning disinfection products and the installation of thermometers in the rooms.

Our proposals mentioned above and presented in the action plan have unfortunately not been applied to 100%, especially those requiring a relatively large budget, we have even found some obstacles with the managers of the centers and cooperatives for the application of good hygiene practices. These continuous trainings that we have carried out have given good results for the hygienic and microbiological quality of raw milk.

Physicochemical and microbiological analysis of raw milk from centers and cooperatives after improvement

The results of physicochemical analyses obtained reached the objective of approaching the target criterion set by the Moroccan regulations. Concerning the TAMF, 89% of the initial load has been reduced. However, some collection centers have not yet completed the corrective actions. This is unfortunately due to the lack of collaboration of some people who are not interested in good hygiene practices. The total coliforms present a high load compared to the initial state, but only in two cooperatives. In fact, the presence of coliforms is not necessarily a direct indication of fecal contamination. Some coliforms are, in fact, present in the wet residues encountered in the dairy equipment. Therefore, it is necessary to have a monitoring and control of hygiene within these cooperatives and centers after the improvement of all axes. Indeed, according to [9], the high rate of bacterial contamination of milk can present health risks for consumers. Most importantly, when not handled properly and under good hygienic conditions, it can promote the growth of pathogenic bacteria and therefore increase the possibility of transmission of foodborne diseases that can compromise the health of the population.

CONCLUSION

Our study has demonstrated the degree of hygienic cleanliness of “raw cow’s milk” from different cooperatives and centers in Beni Mellal-Khenifra region that supply the industry studied in raw milk. Indeed, the physicochemical and microbiological results after improvement become in conformity with the national and international standards except for some exceptions in certain cooperatives and centers, which do not respect the hygiene practices, and the fact that the majority of their managers do not have a level of instruction allowing them a better management. Thus, at the end of this work, we suggest to follow the following recommendations to have good quality milk: Establish a quality policy with the popularization of good breeding practices and insist on the cleanliness of the animals, their immediate environment and the healthiness of the milking. In addition, the dissemination of a notice recommending to the population to boil the local milk before any consumption should be done.

Conflict of interest

The authors declare no conflict of interest.

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