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Microbiological quality of bovine milk from the dairy basin of Relizane for cheese processing .

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ABSTRACT

The prior knowledge of the native microflora of milk, in order to favour useful flora and inhibit pathogenic flora, has become one of the key factors in the control of cheese quality. In this context, an approach to evaluate the bacterial flora of collected cow's milk was tested in the dairy basin of Relizane, in the Dahra region, in three cow's milk producing communes: Djidiouia, Mazouna and Zemmoura, by counting, over a period of high lactation, the total flora, the lactic flora and the pathogenic flora. This assessment will allow us to determine and characterize the bacterial ecosystem at the collection of milk necessary for the orientation of heat treatments in pre-processing and the adaptation of cheese technology at the industrial pole of Sidi-Saada. The results of the microbiological control gave the classification of the milk in class C of unsatisfactory hygienic quality by the presence of a total flora exceeding 106 cfu/ml and the dominance of lactic flora by a majority presence of 72% of the presumed species of Lactococcus and 28% of Enterococcus, and a pathogenic flora dominated by 99% by total coliforms and 1% by Staphylococcus. Prior knowledge of the native lactic microflora of the milk has become a necessary orientation for the cheesemaker, which allows on the one hand the orientation of the treatment and processing techniques and on the other hand to preserve the typicality of the cheeses manufactured.

1.Introduction

The production of cow's milk often comes up against the problem of quality management, which penalizes both producers and processors. Hygiene conditions on farms and the maintenance of the cold chain throughout the production circuit up to the dairies are all sources of contamination that need to be controlled in order to preserve the hygienic quality of the milk necessary for a directed dairy technology (Aggad *et al., 2009;* Faye and Loiseau, 2002; Ghazi and Niar, 2011; Labioui, 2009; Mennane *et al.,* 2007; Srairi *et al.*, 2005 and Yuan *et al.*, 2022).

The processors, in their quality approach, are convinced of the need to involve their producer and collector partners in the application of good production practices in order to improve the quality of the raw milk received. The importance of the native quality concept of the raw material, in this case raw milk, is considerable in the elaboration of derived products typical of each region (Montel *et al.*, 2014; Vignola and Amiot, 2002).

These aspects of improving the quality of raw cow's milk by preserving the original bacterial flora have, however, been little studied. The specificities of the context of cattle breeding in Algeria, dominated mainly by the fluctuation of the hygienic quality of milk oriented towards the cheese industry, imposed the conduct of this study whose main objective was the appreciation of the native microflora of milk by the characterization of the main contaminants that affect the bacteriological quality of raw cow's milk at the level of the Relizane dairy basin, of the 03 communes with a great production potential; Djidiouia, Mazouna and Zemmoura, in order to orientate the transformation to an adapted cheese technology.

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2.Material studied, area descriptions, methods and techniques:

2-1. Selection of regions and collection of milk samples

Random sampling was carried out during the high lactation period (from March 2022 to June 2022) around the major milk production centre, in the dairy basin of Relizane, in the Dahra region Thus, the sampling areas concern the three milk-producing communes of Reizane: Djidiouia, Mazouna and Zemmoura. Forty-eight samples of raw milk were taken at a rate of 4 samples per month per potential collector in each commune.

The milk samples were collected aseptically in sterile containers and kept at low temperatures until the experimental analyses were carried out in the laboratory.

2-2. Microbiological analyses : Research and enumeration of contamination germs :

Different dilutions with tryptone salt solution (TSE) were used depending on the nature of the sample; they varied between 10^{-1} and 10^{-8}.

For each sample, five groups of bacteria were searched for: total aerobic mesophilic flora, faecal streptococci, faecal coliforms, *Staphylococcus aureus*, *Clostridium* sulphite-reducers and technological flora (De Reu *et al.,* 2004).

Total aerobic mesophilic flora (FMAT) was tested and enumerated on plate count agar (PCA) after incubation at 30 °C for 72 h (F.I.L, 2018).

Total and faecal coliforms (CT) and (CF) were tested and enumerated on crystal violet and neutral red bile agar (VRBL), incubated 24 h at 44 °C. All red (lactose +) colonies with a minimum diameter of 0.5 mm that appeared were considered faecal coliforms (JORA, 2017).

Staphylococcus aureus (SA) were tested and counted on Baird Parker agar supplemented with egg yolk and potassium tellurite and incubated at 37 °C for 24-48 h. Colonies appeared black, shiny, convex and surrounded by a clear halo approximately 2-5 mm in diameter. Confirmation was done by Gram stain (+) and testing for catalase (+) and coagulase (+) (Baazize, 2005).

Faecal streptococci (SF) were enumerated on Rothe's medium (Institut Pasteur, Algeria). One millilitre of each test sample was added to 9 ml of TSE broth. This gave a stock dilution of 10^{-1} from which decimal dilutions were made. Then, one millilitre of each dilution was placed in three tubes of Rothe's presumptive medium. After incubation for 48 h at 37 °C, the contents of the positive tubes, i.e. showing cloudiness, were then plated onto bile, esculin and sodium azide (BEA) agar used for confirmation and incubated at 37 °C for 24 and 48 h (F.I.L, 2018).

For *Clostridium* sulphite reductans (CSR) at 46°C on Meat and Liver VF medium supplemented with iron alum and sodium sulphite, an aliquot of milk placed in a sterile tube was previously heated for 10 min at 80°C in order to destroy the vegetative forms and activate the spores. Then, using a sterile pipette, 1 ml of the test sample (milk heated for 10 min at 80 °C) was placed in the VF selective medium (Baazize, 2005).

For the technological flora, inoculation was carried out from the different dilutions by spreading 100µL of the dilutions made on MRS agar and M17 agar. The cultures thus produced were incubated at 37°C for 24 to 72 hours (Gusils *et al.,* 2010).

3. Results and discussion

With regard to the criteria required by the Official Gazette of the Algerian Republic N°39 of 02/07/2017, relating to the microbiological specifications of raw milk, the results obtained during the analysis of the 48 samples are summarized on the table as follows:

Tab. 1. microbiological analysis

		Milk collected from the communes			Average
		Djidiouia	Mazouna	Zemmoura	
FMAT CFU/ml		$2\ 10^6$	$5\ 10^6$	$1,2\ 10^6$	$2,7\ 10^6$
CT CFU/ml		$2\ 10^2$	$1,5\ 10^4$	$1,6\ 10^2$	$5,6\ 10^3$
CF CFU/ml		0	0	0	0
SF CFUml		0	0	0	0
SA CFU/ml		0	$1,5\ 10^2$	0	50
CSR CFU/ml		0	0	0	0
Technological flora	***Lactococcus*** CFU/ml	$2\ 10^3$	$5\ 10^3$	$1,8\ 10^3$	$2,9\ 10^3$
	Enterococcus CFU/ml	$8\ 10^2$	10^3	$1,65\ 10^3$	$1,15\ 10^3$

In the light of the results obtained, it appears that the milk is increasingly contaminated as it passes through the various stages of production in the milking parlour, the means used in collection, transport, arriving at the reception of the dairy industry. Thus, from the udder to the milk storage tank, the non-regulation of production and collection practices led to fluctuations in the microbiological quality of the milk studied.

Indeed, this rapid deterioration of the bacteriological quality of the milk throughout the production chain is the result of successive contamination from utensils, udders, teat cups, milking environment, milking hands, milking, collection and transport equipment. It is during the harvesting process that milk becomes contaminated, and the more it is handled, the greater the risk of bacterial contamination.

Bacterial research revealed that milk from the Mazouna region was the most contaminated and carried the germs of interest (FMAT, CT and SA). The collection area of this commune is the largest and the effect of adding several raw milks from different cows contributes to the lower quality of the blended milk (tank milk).

In addition, *Staphylococcus aureus* was present in the Mazouna milks and is a contagious agent living on the cow's udder and is transmitted from cow to cow (Meskini *et al.,* 2021). This bacterium can enter the milk either by direct excretion from udders with clinical or sub-clinical staphylococcal mastitis or by environmental contamination during handling and processing of raw milk (Mennane *et*

al.,2007). When the udder is infected,

Staphylococcus aureus is excreted in milk in amounts that have shown wide fluctuations, from 0 to $1.5.10^2$ CFU/ml. These results, which are in agreement with those of Meskini *et al* (2021), suggest that the bacterium may originate mainly from the water used during the different milking steps, from the hands of the milkers and from the udders.

The presence of total aerobic mesophilic flora (FMAT) in raw milk provides information on the overall hygienic quality of the farm. FMAT includes spoilage or contamination microorganisms, acidifying lactic flora and sometimes pathogenic bacteria. The enumeration of this flora is the most common method used by milk processing units to assess the bacterial quality of milk and is therefore an important indicator of hygiene conditions during milking (Aggad *et al*., 2009 and Yuan *et al*., 2022). The high presence of this flora in milk samples from refrigeration tanks is probably the result of intense bacterial multiplication, favoured by the lack of hygienic conditions during milking and milk storage. The average enumeration obtained classifies our milk in category C of more than 10^6 CFU/ml.

The presence of total faecal coliforms in raw milk indicates an environmental source of contamination. Their abundance in raw milk reflects the non-observance of sanitary provisions required during milking for milk collection, and probably contamination during milk storage. The main vectors are strongly linked to the teat skin, soiled by faeces and poorly designed and cleaned milking equipment (Baazize, 2005). Indeed, Meskini *et al* (2021) were able to report that poor cleaning of milk contact containers on the farm leaves residual levels of contamination.

Technological flora

Lactic acid lactococci play an essential role in the production of fermented milk and cheese (Dahou *et al.,* 2015). They can be present as wild lactic acid bacteria, natural elements in the milk flora, brought in by teats, milking and milk storage equipment and the manufacturing plant, or deliberately seeded into the milk. *L. lactis* is a model bacterium for fundamental research and has been the subject of numerous studies for several decades. It is also the first lactic acid bacterium whose genome has been completely sequenced.

Enterococci are lactic acid bacteria that have been used for centuries in food processing. These micro-organisms play an essential role in the preservation (extension of storage time) and bacteriological quality of foods, while respecting their nutritional and organoleptic properties. However, they are markers of faecal contamination (*Enterococcus faecalis* and *Enterococcus faecium*) and are also involved in the development of nocosomal diseases. The genetic plasticity (transfer of genetic elements) of these bacteria allows them, on the one hand, to adapt to many ecosystems and, on the other, to be vectors of antibiotic resistance and bacterial virulence (Faye and Loiseau, 2002). Consequently, the use of enterococci in the food industry is increasingly controversial. Involved in the fermentation of many foods (milk, vegetables, meat and fish), enterococci are capable of producing various antimicrobial molecules (lactic acid, bacteriocin or hydrogen peroxide). These properties make them indispensable to the food industry. When used as protective micro-organisms, they must be carefully characterised and studied in advance to demonstrate their safety.

4. Conclusion

This study shows that the increase in the bacterial load of milk throughout the production chain on the farm is the result of successive contaminations associated with poor hygiene practices during milking. The search for the sources of contamination at the collection of raw milk showed that the non-regulation of production practices is the source of contamination of milk by the bacteria of interest. To improve the quality of raw milk, various hygiene measures in the stables and during milking must be applied by farmers, all the more rigorously and systematically as the environment of the animals is highly contaminated. Reducing this environmental contamination requires the implementation of manure storage and spreading practices to avoid the recycling of bacteria and their dissemination. This is difficult to achieve without the effective participation of farmers and prior information efforts for them. The hygienic quality of the milk and the stability of its constituents give rise to oriented processing, a very wide range of dairy products and controlled typicality.

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