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Assessment of physico-chemical and microbiological quality of raw cow milk from Relizane area, Algeria.

MESKINI Zakaria[1]*, HOMRANI Mounia[1], DAHOU Abdelkader El-Amine[1], YEROU Houari[2], BELKACEM Amina[1], HOMRANI Abdelkader[1]

1 Laboratory of Animal Production Sciences and Techniques, University of Abdelhamid Ibn Badis Mostaganem, 27000, Algeria.

2 Laboratory of Geo-sciences and sustainable development. University of Mascara, 29000, Algeria.

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ABSTRACT

As a key component of the daily diet of Algerians, milk plays an important role in meeting rising nutritional demands while also ensuring food safety in developing nations such as Algeria. The objectives of this study were to evaluate the physico-chemical and microbiological quality of raw milk and to investigate the dairy cow husbandry practices. The raw milk quality was evaluated in three times. The results showed that raw milk was characterized by a moderate physico-chemical proprieties, fat cotenant ranged between 31.5 to 31.9 g/l and protein level was between 30 to 32.5 g/l. The pH ranged between 7.5 and 7.6. The ranges of lactose (47.1 to 59.7), minerals (6.8 to 8.8), Density (1.031 to 1.032), Dry Degreased Extract (99 to 114.3) were observed. While the raw milk samples were of poor microbiological qualities. Total mesophilic aerobic flora was high in the third sample 1.13×10^6 cfu/ml. Fecal coliforms ranged between 3.77×10^3 and 2.28×10^4 cfu/ml. However, the staphylococci were absent in all samples. The Montbéliarde was the main breed. Dairy cows were keptin a freestall barnand feed with a total mixed ration. The average milk production per cow per day was 28 liters. The findings of this research indicated that the raw milk has a good physico-chemical qualities where as the microbiological quality was under the standards. Indeed, there is a necessity to follow a stricter milking hygiene procedure to produce a raw milk that satisfies quality requirements.

1.Introduction

Milk plays an important role in food and nutrition policy in daily life in Algeria. Furthermore, milk is an important meal due to its nutritional value, which provides a large contribution in protein, lipids, and minerals. The average yearly milk consumption per capita is 96,96 kg (Ramdane *et al.*, 2019). Physico-chemical qualities, microbiological quality, nutritional values, sensory properties, technical compliance establish raw milk quality in accordance with consumer needs. Consumers place a high value on milk quality and safety (Hamiti *et al.* 2014). As a result, ensuring good quality and ideal physico-chemical qualities of raw milk is difficult since they are dependent on numerous factors including the feed, health, milking method, hygiene, breed, and season, which are all important factors in milk quality.

Hygienic procedures are necessary to reduce the microbial population and minimize new intramammary infections because the teat surface is a potential direct source of microorganisms to the milk (Vacheyrou *et al.*, 2011; Verdier-MeTz *et al.*2012). It is reported by BöhM *et al.* (2017), that the microbial load is lower on the skin of teats disinfected prior to milking compared to teats that are only cleaned. There are many sources of microorganisms that can contaminate teats including, wash water, bedding, soil, hands, milking equipment, contaminated milk and udders (Philpot, 1984).

Cows are kept and managed using various farming practices. Control of animal health, adherence to excellent milking systems, and control of milking parlor clean liness are critical in decreasing the microbial load in raw milk (Bekuma and Galmessa, 2018). Proper housing, nutrition, and equipment assist guarantee that the animals are properly cared for and that enough facilities can handle the cows successfully.

*Corresponding author.

E-mail address: meskinivet@gmail.com

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The purpose of this research was to investigate the physico-chemical proprieties of dairy raw milk by evaluating the fat and protein rate, freezing point and density, and the microbiological quality, by targeting the total mesophilic aerobic flora, staphylococcus aureus, fecal and total coliform. In addition, the management practices of dairy cows farming were assessed.

2.Material and methods

2-1. Milk analysis

2-1-1. Sampling method : The collection of the samples took place at dairy farm in Relizane region, Algeria. To avoid external contamination, raw milk samples were obtained from cooling tank using a ladle ignited with alcohol and placed into sterile bottles. The samples of raw milk were taken in three periods spaced with 2 weeks, 25 April; 09 may; 22 may 2022. In each time two samples were obtained; the first sample of 50 mL was for physico-chemical analyses. While the second sample of 100 mL was for the microbiological quality. Samples were kept and transferred immediately after collection in an isothermal container at 4°C, and the analyses were carried out within three hours.

2-1-2. Physico-chemical analysis: The physico-chemical analyses were performed by Lactoscan milk analyzer to determine physico-chemical qualities. standard parameters were measured such as fat, protein, density, mineral matter, dry matter, freezing point and lactose.

2-2. Microbiological analysis

2-2-1. Dilution preparation: Raw milk sample were homogenized and dilutions up to 10-6 were prepared in accordance with the ISO standard (NF EN ISO 6887-1) which establishes the general guidelines for the preparation of suspension samples and decimal dilutions for the microbiological inspection of food. The 0.1% peptone water was utilized as a diluent.

2-2-2. Bacterial enumeration: Total aerobic mesophilic flora enumeration was performed on Plate Count Agar media according to ISO 22301 standard and incubated for 24 to 72 hours at 30°C. The enumeration of fecal coliforms was performed on a selective medium Violet Red Bile lactose Agar (VRBL) according to ISO 4832 standard. The incubation period was for 24 hours at 30°C. The enumeration of total coliform was carried out on a selective medium Violet Red Bile Glucose Agar (VRBG) according to ISO 21528-2 guidelines. The incubation period was for 24 hours at 30°C. The enumeration of *Staphylococcus* aureus was performed on Chapman medium and incubated for 24 hours at 37°C.

2-3. Dairy cow management

A survey was carried out including in total 60 cows. A visit of the productive environment and an interview with the farmer were done through a questionnaire that included different sections; socioeconomic (name, status, number of employees, utilized agriculture area), livestock (number of animals, breeds of cows), housing system (number of buildings, type of housing), reproduction management, feeding management, milking system.

3. Results and discussion

3-1. Physico-chemical analysis

The physico-chemical parameters were measured three times and in triplicate. Table 1 shows the results of the physico-chemical analysis of raw milk. In our study it was found that the fat values were between 31.5 and 31.9 g/l, the highest value was in the third analyze 31.9 g/l and the lowest value was in the first analysis 31.5 g/l. It was also observed that the protein of raw milk samples ranged between 30.8 and 34.9g/l. The pH of raw milk was between 7.51 and 7.3. The sample tested were found to have a density 1.032. Concerning lactose content, the values were different in each analysis and the highest value were recorded in the second analysis.

Tab. 1. Raw milk physico-chemical parameters .

Parameters	1st Analyze	2nd Analyze	3rd Analyze
Fat content (g/l)	31,5	31,75	31,9
Protein content (g/l)	30 ,8	34,9	32,5
Density	1,032	1,031	1,032
Minerals	6,8	8,8	7,5
Lactose (g/l)	47,1	59,7	51,7
Dry Degreased Extract (g/l)	99,9	114,3	106,3
Freezing point (°C)	-0,54	-0, 74	-0, 61
pH	7,63	7, 54	7, 51

The fat content of milk is an essential milk quality criterion since it impacts its nutritional value. However, the most variable component of bovine milk is fat. The fat content of milk changes mostly depending on food and lactation stage. Fat content of raw milk was lower in comparison to the Algerian standard which should be 34 g/l. Also, it was lower than that recorded in Guelma region 37.2g/l (Bousbia *et al.,* 2018) and in Djelfa region which was on average 36.6 g/l (Hamiroune *et al.*, 2019).

The freezing point of raw milk is constant, with breed, lactation stage, and season very slightly influencing it. Although somewhat bigger differences might be predicted if cows are underfeeding or do not have free access to drinking water (Bhandari and Singh, 2011), the freezing point test is extensively used to identify water adulte ration in milk, because it is proportional to the amount of water in milk. In Algeria, a freezing point of -0.51°C or below is typically considered as the norm for unadulterated raw milk. In our study the freezing point was in accordance with Algerian norms and ranged between -0.54 to -0.74°C.

Protein content of milk was slightly higher than the value of raw milk collected in the Guelma region, which was 29.42 g/l (Bousbia *et al.,* 2018), and in similar to raw milk analyzed in the nord-east of Algeria which was 32.8 g/l (Matallah *et al.,*2017).

Lactose is the primary sugar found in milk and serves as the substrate for lactic fermentation. The rate of lactose in our study was higher than recorded in the study of Bousbia *et al.* (2018) which was 44.1 g/l on average.

Measurement of milk pH is crucial in testing for impurity, degradation, and mastitis infection signs. Fresh milk has a pH of roughly 6.7. When the pH of the milk falls below 6.7, it often deteriorates due to bacterial decomposition. Overall, the pH values recorded were higher, ranging between 7.51 and 7.63, than the standards of 6.7.

One of the key approaches for determining milk adulteration is determining its density. The density of milk changes depending on the action of all elements in its composition. While increasing fat content reduces density, increasing protein, lactose, and mineral content and/or lowering fat content increases density. The value of den-

sity was in agreement with the Algerian standards of 1.028 to 1.033.

3-2. Microbiological analysis

Microbiological results showed in table 2 indicate that the total aerobic mesophilic flora count was high and above standards, with a high value recorded in the first analysis 8.19×10^5. The fecal coliform count varied between the three analyses; the first analyze showed an absence of fecal coliform, where as in the second and third analyses was highly contaminated. Concerning the total coliform count was quite high in all three analyze. It should be noted that the staphylococci were not found in all examined raw milk samples.

Tab. 2. description of different flora enumeration.

Flora	1st Analyze	2nd Analyze	3rd Analyze
TMAF	$8{,}19\times10^5$	$2{,}41\times10^5$	$1{,}13\times10^6$
Fecal coliform	Abs	$3{,}77.10^3$	$2{,}28\times10^4$
Total coliform	$1{,}91\times10^5$	$3{,}49\times10^4$	$3{,}3\times10^5$
Staphylococcus aureus	Abs	Abs	Abs

The presence of numerous pathogenic microorganisms can be indicated by a high total bacterial count in raw milk, and ingestion of pasteurized or boiling milk from this milk may result in the swallowing of heat-resistant toxins from microorganisms. The large overall quantity of microorganisms not only endangers human health, but also generates significant economic losses in the region. The Algerian legislation considers that a load greater than 3×10^6 cfu/ml indicates significant contamination.

The TMAF informs on the global quality of milk, the temperature of conservation, as well as the level of hygiene. In our study the TMAF was in the limit of the acceptable level by the Algerian legislation. In addition, the TMAF was similar with other study conducted in Algeria, Aggad et al. (2009) reported an average of 83 x 10^4 and Bachtarzi *et al.* (2015) reported an average of 28.8×10^6 CFU ml-1.

The absence of fecal coliforms in the first assay suggests that milking hygiene was good. However, in the second and third analyses, they are greater than the Algerian norms of 10^3 CFU ml-1. The presence of fecal coliforms is thought to be an indicator of fecal contamination; hence it is more of a sign of inadequate hygiene management and contamination of milk by manure or insufficient milking cleanliness. Indeed, the fecal coliform in our study was higher than the average of 170 cfu ml-1 reported by Ghazi and Niar (2011) in the region of Tiaret, and lower than that recorded in the study of Sassi et al., (2018) in Relizane area (5.2×10^3 cfu ml-1).

Coliform count is critical in the dairy products since coliforms represent contamination in milk, soil, and water, as well as inadequate hygiene procedures. As a result, several nations have set legal limitations for the presence of coliforms in milk and dairy products. Total coliform enumeration results varied significantly between samples, with a load of 3.3×105 exceeding the required standard of 103cfu/g. These findings are higher in comparison with the study of Matallah *et al.* (2017) who reported a load of $4{,}7\times10^4$ cfu/mL. These bacteria are sensitive to heat, and their presence is linked to fecal contamination.

It should be emphasized that all three milk samples were negative to staphylococci. This implies appropriate sanitary behavior at the time of sampling procedures as well as the animal's overall health. The presence of staphylococci might be attributed to the prevalence of staphylococcal mastitis in dairy farms. In addition, it was reported that staphylococcus are the main bacterial species responsible of mastitis (Meskini *et al.*, 2021b).

3-3. Dairy cow management

The survey included 60 dairy cows, 15 heifers, 17 calves, and 2 bulls. The studied farm had two buldings. The first for cows and heifers, while the second was for calves and bulls. Natural light and ventilation were used inside the buildings, and during the hot season the farmer used a mechanical ventilation to minimize high temperature in the building. The housing system used was the Freestall system, and a rubber mat was used as bedding for the cows.

The farm feed dairy cows with a total mixed ration, dairy cow feed included corn silage with 30 kg, concentrate feed 8 kg, and straw 2 kg. The milking system was mechanical, cows were milked twice a day in milking parlor. The daily milk production of dairy cows was on average 28 l per cow. Farmer used natural service as a main method to inseminate cows, farmer detects heat through direct observation. Concerning heifers, they were accepted on breeding program when they reach a weight of 450 kg, which was usually around 12 months of age.

The farm overall utilized agricultural area was 2 ha, which is less than the 7.47 ha recorded in the same region (Meskini *et al.*,2020). Concerning the cow population was higher than the average recorded in Mostaganem 11.44 heads (Meskini et al.,2022). Montbeliarde was the main breed on farm, in difference with the study of Yerou et al. (2019) who reported that the Holstein was the main breed in western Algeria. Like the majority of farmers in Algeria natural service was the main breeding method used (Benidir *et al.*, 2020; Meskini et *al.*, 2021a).

4. Conclusion

The results of our study show an overview of the physico-chemical, microbiological quality of raw milk and furthermore cattle management.The raw milk samples were found to have a moderate nutritional quality, as well as an acceptable level of total mesophilic aerobic flora, absence of staphylococci. However, the total and fecal coliform were above the limit authorized by the legislation of our country. The dairy cow management has several critical points, absence of forage area, use of only natural service and a bad estrus detection system. Our results revealed that the raw milk can present a potential risk for consumers, the hygienic quality of raw milk must be considerably improved. Hygienic procedures such as pre-dipping, forstripping and post-dipping, wearing gloves while milking, and adhering to milk storage conditions should be updated to meet the standards hygienic rules in dairy farms .

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