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Physicochemical and microbiological status of raw milk in a semiarid region of Algeria

Calidad fisicoquímica y el estado microbiológico de la leche cruda en una región semiárida de Argelia

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Zakaria Meskini[1,2*], Abdelkader El-Amine Dahou[2], Amina Derradjia[3] and Elhachemi Sassi[2,4]

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ABSTRACT

Milk is an important component of human nutrition and food security in Algeria, although, its quality may be affected by inadequate hygienic and handling practices. This study evaluated the physicochemical and microbiological quality of raw milk and examined regional variability in a semi-arid area of northwestern Algeria. A total of 42 bulk tank milk samples were analyzed using standard ISO methods and Lactoscan-based compositional analysis. Physicochemical parameters including fat, protein, lactose, solids-not-fat, density, mineral content, freezing point, and pH were compared against established reference criteria. Microbiological analyses encompassed total viable counts, total and fecal coliforms, and *Staphylococcus* spp. Significant regional differences were observed in several milk quality parameters. Mean fat (2.94%) and lactose (4.44%) contents were frequently below recommended reference values, whereas solids-not-fat content (8.48%) generally complied with established standards. The mean TVC reached 5.45 log CFU mL^{-1}, while total and fecal coliforms count averaged 2.54 and 3.12 log CFU mL^{-1}, respectively. Elevated levels of *Staphylococcus* spp. were detected in three regions. Correlation analyses showed significant associations between microbial indicators and certain compositional parameters, particularly pH. Overall, the results indicate substantial regional variability in raw milk quality and suggest deficiencies in hygienic conditions during milk production and handling. These findings highlight the importance of improving milking hygiene, refrigeration practices, and quality control measures to enhance raw milk quality in semi-arid dairy production systems.

Palabras clave:
Composición química
Granjas lecheras
Higiene alimentaria
Perfil nutricional
Contaminación patógena

RESUMEN

La leche cruda es un componente importante de la nutrición humana y la seguridad alimentaria en Argelia, aunque su calidad puede verse afectada por prácticas higiénicas y de manipulación inadecuadas. Este estudio evaluó la calidad fisicoquímica y microbiológica de la leche cruda y examinó la variabilidad regional en una zona semiárida del noroeste de Argelia. Se analizaron un total de 42 muestras de leche de tanque de refrigeración colectivo mediante métodos estándar ISO y análisis de composición con Lactoscan. Los parámetros fisicoquímicos incluyeron grasa, proteína, lactosa, extracto seco magro (ESM), densidad, contenido mineral, punto de congelación y pH. Los análisis microbiológicos incluyeron el recuento total de microorganismos viables (RTV), coliformes totales, coliformes fecales y *Staphylococcus* spp. Se observaron diferencias regionales significativas en varios parámetros de calidad de la leche. Los contenidos medios de grasa (2,94%) y lactosa (4,44%) se situaron frecuentemente por debajo de los valores de referencia recomendados, mientras que el contenido de extracto seco magro (8,48%) cumplió, por lo general, con las normas establecidas. El RTV medio alcanzó 5,45 log UFC mL^{-1}, mientras que los recuentos de coliformes totales y fecales promediaron 2,54 y 3,12 log UFC mL^{-1}, respectivamente. Se detectaron niveles elevados de *Staphylococcus* spp. en tres regiones. El análisis de correlación mostró asociaciones significativas entre los indicadores microbiales y ciertos parámetros de composición, particularmente el pH. En conjunto, los resultados indican una variabilidad regional sustancial en la calidad de la leche cruda y sugieren deficiencias en las condiciones higiénicas durante la producción y manipulación de la leche. Estos hallazgos resaltan la importancia de mejorar la higiene del ordeño, las prácticas de refrigeración y las medidas de control de calidad para optimizar la calidad de la leche cruda en los sistemas de producción lechera de zonas semiáridas.

[1]Department of Agricultural Sciences, Laboratory of Environment, Natural Plant Substances and Food Technology, University of Ahmed Zabana Relizane, 48000 Relizane, Algeria. meskinivet@gmail.com
[2]Laboratory of Sciences and Techniques of Animal Production, Faculty of Natural and Life Sciences, Abdelhamid Ibn Badis University, 27000 Mostaganem, Algeria. abdelkader.dahou@univ-mosta.dz
[3]Department of Biological Sciences, Faculty of Natural and Life Sciences, University of Ahmed Zabana Relizane, Relizane, Algeria. amina.derradjia@univ-relizane.dz
[4]Department of Nutrition and Food Technology, Laboratory of Animal Production Sciences and Techniques, University of Ibn Khaldoun Tiaret, 14000 Tiaret, Algeria. elhachemi.sassi@univ-tiaret.dz
*Corresponding author

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Milk is a fundamental component of human nutrition due to its high biological value and balanced content of proteins, lipids, lactose, minerals, and vitamins. In Algeria, milk constitutes a strategic food commodity with an annual per capita consumption estimated at approximately 97 kg, highlighting its nutritional and socioeconomic importance (Ramdane et al. 2019). Beyond availability, raw milk quality and safety have become a major challenge for public health and dairy sustainability.

Raw milk quality is commonly evaluated through physicochemical composition and microbiological status, both of which are important indicators of hygienic production conditions and compliance with regulatory standards. The Codex Alimentarius Commission establishes guidelines for raw milk hygiene, including acceptable microbial loads and compositional requirements (CAC 2004).

Physicochemical parameters such as pH, density, fat, protein, and freezing point are widely used to assess milk composition, technological properties, and potential adulteration. At the same time, microbiological indicators, including total viable counts and coliform bacteria, provide valuable information regarding milking hygiene, storage conditions, and sanitary practices during milk handling.

The physicochemical and microbiological characteristics of milk are closely interconnected. Alterations in milk composition may result from microbial activity, inadequate handling, or adulteration practices, whereas poor hygienic conditions can accelerate bacterial proliferation and compromise milk stability and quality (Baran and Adıgüzel 2020).

Therefore, integrated evaluation of physicochemical and microbiological parameters is essential for a comprehensive assessment of raw milk quality.

Microbial contamination of raw milk occurs mainly at the farm level, and may originate from the teat surface, housing conditions, milking equipment, water quality, or personnel hygiene (Meskini et al. 2021a). Consequently, inadequate milking hygiene and weak herd health management are associated with elevated bacterial counts and increased risk of mastitis, leading to deterioration of milk quality and economic losses (Bekuma and Galmessa 2018).

In Algerian dairy production systems, particularly small and medium scale farms continue to face challenges related to hygienic management and structured herd health programs (Meskini et al. 2023). Although several studies have investigated specific aspects of milk quality, comprehensive assessments integrating physicochemical composition, microbiological quality, and regional variability under local production systems remain limited especially in semi-arid regions.

Therefore, the present study aimed to evaluate the physicochemical and microbiological quality of raw cow's milk collected from different geographical areas of Relizane governorate in Algeria, in accordance with national and international standards. The study also examined the relationships between compositional and microbiological indicators as potential markers of hygienic conditions and milk handling practices.

MATERIALS AND METHODS

Study area

The study was conducted in the province of Relizane, located in north-western Algeria. The region is characterized by a Mediterranean climate with continental influence. Mean annual temperature ranges from 17 to 19 °C, with summer maxima exceeding 35 °C, while mean annual precipitation varies between 350 and 450 mm and occurs mainly from October to March (Attou et al. 2025). Relizane is an important dairy production area dominated by small to medium scale farms (Meskini et al. 2020).

Farm selection criteria and study design

A cross-sectional study was conducted to evaluate the physicochemical and microbiological quality of raw cow's milk. Dairy farms were selected using purposive sampling based on the following inclusion criteria. Presence of a functional bulk milk tank; lactating cows milked at least once daily; absence of antibiotic treatment during the sampling period; willingness of farmers to participate. Farms with clinical mastitis outbreaks or recent chemical sanitization of bulk tanks (<48 h before sampling) were excluded.

The required sample size was initially estimated using Yamane's formula for finite populations (Yamane 1967), as shown in Equation 1.

$$n = \frac{N}{1 + N(e)^2} \quad (1)$$

Where n is the required sample size, N is the total population size, and e is the margin of error.

The calculation indicated a minimum sample size of 75 farms at a 10% margin of error. However, due to logistical constraints, limited farm accessibility, and non-eligibility of several farms during the sampling period, only 42 farms meeting inclusion criteria were ultimately enrolled in the study.

A total of 42 bulk tank milk samples were collected, with each farm sampled once. Samples were distributed across four districts as follows: Oued El Djemaa (n = 6), Zemmora (n =16), Oued Rhiou (n = 6), and Relizane (n = 4).

Sampling period

Milk sampling was conducted over a five-month period from March to July 2025 to capture routine production conditions across the study area. Each dairy farm was sampled once during the study period. Extending sampling over several months allowed greater operational flexibility and reduced temporal clustering of sample collection.

Milk analysis

Sampling method

Raw milk samples were collected aseptically from bulk tanks following ISO – 707 recommendations (ISO 1995), milk was thoroughly homogenized by agitation. Approximately 60 mL of milk was collected using a sterile stainless-steel ladle that had been flamed and cooled prior to use. Each sample was divided into two sterile bottles, one for physicochemical analysis and one for microbiological analysis. Samples were immediately placed in insulated containers with ice packs ($\leq$4 °C), transported to the laboratory within 6 h, and stored at 4 °C. All analysis were completed within 24 h of collection.

Physicochemical analysis

Physicochemical analyses were performed using a Lactoscan ultrasonic milk analyzer (Milkotronic Ltd., Nova Zagora, Bulgaria). Prior to analysis, samples were equilibrated to 20±1 °C according to the manufacturer's recommendation. The following parameters were measured: Fat (%); Protein (%); Lactose (%); Density (g L^{-1}); Solids-not-fat (SNF, %); Freezing point (°C); Ash content (%) expressed as mineral matter. All measurements were performed in triplicate, and mean values were retained for statistical analysis. Results were compared against compositional standards and international reference standards for raw cow milk.

Microbiological analysis

Sample preparation

Serial decimal dilutions were prepared in accordance with ISO 6887-1 (ISO 2017). Briefly, 1 mL of milk was homogenized in 9 mL of sterile 0.1% peptone saline solution (10^{-1}), followed by successive dilutions up to 10^{-7}.

Bacterial enumeration

Total viable count were determined using the pour-plate method on Plate Count Agar (PCA) according to ISO 4833-1 (ISO 2013), incubated aerobically at 30 °C for 72 h. Total coliforms were enumerated on Violet Red Bile Lactose Agar (VRBL) following ISO 4832 (ISO 2006), incubated at 37 °C for 24 h. Fecal coliforms were determined on VRBL agar incubated at 44 °C for 24 h, in accordance with ISO 11866-2 (ISO 2005). *Staphylococcus* spp. were enumerated using Mannitol Salt Agar, incubated at 37 °C for 24-48 h (ISO 6888-1/2) (ISO 2021). All microbiological analysis were inoculated in triplicate and results were expressed as log CFU mL^{-1}.

Statistical analysis

Microbiological counts were log transformed prior to analysis. Statistical analyses were conducted using IBM SPSS Statistics v26.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro-Wilk test whereas homogeneity of variances was evaluated using Levene's test. Depending on data distribution and variance homogeneity, comparisons among regions were performed using one-way ANOVA or Welch's ANOVA, followed by Games-Howell post hoc tests when appropriate. For non-normally distributed physicochemical variables, regional comparisons were performed using the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction. Comparisons between observed values and reference standards were conducted using the one-sample Wilcoxon signed-rank test. Associations between microbiological counts and physicochemical parameters of raw milk were evaluated using Spearman's rank order correlation (Spearman's ρ). All statistical tests were conducted at a significance level of $P<0.05$.

RESULTS AND DISCUSSION

Physicochemical analysis

The mean fat content across the study regions was 2.94% (range, 2.06-3.18%) and the median value differed significantly from the reference standards (P<0.001). Only Oued El Djemaa (3.17%) and Oued Rhiou (3.18%) approached the recommended reference value of 3.5% (EU N° 1308) (EU 2013), whereas Zemmora (2.06%) and Relizane (2.94%) exhibited lower fat contents (Table 1). The overall mean for solids-not-fat (SNF) was 8.48% (range, 8.0-10.68%). Although the average SNF value approximated the international reference criterion of 8.5% (Azhar et al. 2024), samples from Oued Rhiou and Relizane (both 8.0%) remain slightly below the recommended level.

Mean protein content (3.1%) was within the European regulation of >2.9% (EU N° 1308) (EU 2013). The median protein content differed significantly from the reference value (P<0.001). Although, Zemmora (2.82%) showed the lowest value. Milk density values ($\geq$ 1028 g L^{-1}) aligned with the standard range 1027 to 1033 g L^{-1} (Goff et al. 2021), except for Oued Rhiou (1026.39 g L^{-1}) exhibiting a slight deviation. Average lactose content (4.44%) was slightly lower than the standard physiological range reported for raw bovine milk (4.5-5.0%) (Mahmood and Usman 2010), with the lowest value observed in the Zemmora region (4.15%). In contrast, mean mineral content (0.64%) and pH (6.9) remained within the normal reference ranges reported for fresh bovine milk (Park et al. 2007). Nevertheless, the median mineral content differed significantly from the reference value of 0.7% (P<0.001).

Significant regional variations were observed across all physicochemical parameters of raw cow milk. Zemmora consistently exhibited lower fat, mineral, SNF, freezing point, and lactose compared with Oued El Djemaa (P = 0.006, 0.004, 0.042, 0.010, and 0.002, respectively). Conversely, Oued El Djemaa showed the highest SNF content, significantly exceeding all other regions (P = 0.003, 0.042). Relizane exhibited significantly higher protein than Zemmora and Oued Rhiou (P<0.001), whereas Oued Rhiou exhibited the lowest pH (Table 1). These findings reveal marked spatial heterogeneity in milk composition.

Table 1. Physicochemical milk quality parameters and regional effects.

Parameter	Oued El Djemaa	Zemmora	Oued Rhiou	Relizane	Total Average	P-value*	Standard range
Fat (%)	3.17	2.06	3.18	2.94	2.94±0.73	0.004	3.5%[a]
Density (g L^{-1})	1031.7	1031	1026.39	1028	1028.1±4.15	<0.001	1027-1033[b]
Minerals (%)	0.77	0.59	0.62	0.65	0.64±0.1	0.008	0.7%[c]
SNF (%)	10.68	9.15	8.0	8.08	8.48±1.12	<0.001	>8.5%[d]
Protein (%)	3.27	2.82	2.96	3.32	3.1±0.3	<0.001	>2.9%[a]
Freezing Point (°C)	-0.63	-0.47	-0.52	-0.53	-0.53±0.07	0.008	-0.512 to -0.550 °C[b]
Lactose (%)	5.28	4.14	4.47	4.32	4.44±0.45	0.002	4.5-5.0%[e]
pH	7.5	7.9	6.57	6.68	6.9±0.52	<0.001	6.6-6.9[c]

* Kruskal-Wallis test P-value. [a] European Commission Regulation (EU No. 1308) (EU 2013); [b] Goff et al. (2021); [c] Park et al. (2007); [d] Azhar et al. (2024); [e] Mahmood and Usman (2010).

Significant regional variability in milk composition suggests heterogeneity in dairy production conditions and management practices across the study area. Mean protein content (3.1%) remains within recommended standards, whereas fat content (2.94%) was lower than the international value of 3.5%. Similar reduction in fat content has been reported in other Algerian dairy regions (Mascara region) (Benamara et al. 2024). The lower fat and protein observed in the Zemmora region may reflect differences in feeding practices, forage quality, or herd management conditions. In contrast, higher values recorded in Oued El Djemaa and Relizane may indicate more favorable nutritional or management conditions.

The average freezing point (-0.53 °C) generally complied with the reference of -0.512 to -0.550 °C (Goff et al. 2021).

However, the higher freezing point observed in Zemmora may suggest possible dilution practices or alterations in milk composition, as previously reported in the Steppic region of Djelfa (Hamiroune et al. 2019). Although the mean SNF content (8.84%) was close to the regulatory minimum threshold, it was lower than the considerably higher SNF values (10.46%) reported in Eastern Algeria (Kara et al. 2024). Similarly, geographical differences in lactose concentration may reflect variations in feeding strategies, nutrient availability, and the metabolic efficiency of lactose synthesis in the mammary gland.

Microbiological results

Descriptive statistics for microbiological parameters across the four study regions are summarized in Table 2. Substantial regional variability was observed, with mean values and confidence intervals indicating disparate levels of sanitary quality across the regions.

The TVC ranged from 5.01 to 5.96 log CFU mL^{-1}, with an average of 5.45 log CFU mL^{-1}. This value was not statistically different (P = 0.712) and marginally within the national standard of <5.47 log CFU mL^{-1} (JORA 2017), meanwhile the TVC median was significantly different from the recommended standards (P<0.001). No significant regional differences were observed for TVC (P = 0.512), suggesting relatively comparable background microbial loads across the study regions.

In contrast, total coliform counts exhibited significant regional variation (P<0.001), ranging from 1.89 to 5.11 log CFU mL^{-1} with average value 2.54 log CFU mL^{-1}. Coliform counts in Oued El Djemaa, Zemmora, and Oued Rhiou exceeded the recommended threshold of <2 log CFU mL^{-1} (EU 2021). Oued El Djemaa exhibited significantly higher coliform counts than the other regions, while Zemmora also showed significantly higher levels than Relizane (P = 0.028).

Table 2. Microbiological milk quality (log CFU mL^{-1}) and regional effect.

Region	Oued El Djemaa	Zemmora	Oued Rhiou	Relizane	Total Mean	*P*-Value	Reference criterion
Total viable counts	5.76	5.02	5.96	5.01	5.45	0.512	<5.47[a]; recommended 3-5[b]
Coliforms	5.11	3.81	2.08	1.89	2.54	<0.001	<2[c]
Fecal Coliforms	2.63	0.65	3.71	3.57	3.12	0.014	<2.699[a]; recommended <2[b]
Staphylococcus spp.	0	2.77	3.29	3.49	2.98	/	<2[a]

[a] JORA (2017); [b] Goff et al. (2021); [c] EU (2021).

Fecal coliform loads ranged from 0.65 to 3.57 log CFU mL^{-1}, with an overall mean of 3.12 log CFU mL^{-1} substantially surpassed the national standard of <2.699 log CFU mL^{-1} (JORA 2017). Significant regional variation was observed (P = 0.014). Notably, Zemmora exhibited markedly lower fecal coliform loads compared with Oued Rhiou and Relizane.

Staphylococcus spp. also demonstrated regional variability. No *Staphylococcus* spp. were detected in Oued El Djemaa samples, whereas the remaining regions showed values ranging from 2.77 to 3.49 log CFU mL^{-1}, with a mean of 2.98 log CFU mL^{-1}. Mean counts exceeded the national recommended limit of <2 log CFU mL^{-1} (JORA 2017). However, statistical comparison among regions could not be reliably assessed because of zero variance in Oued El Djemaa.

Total viable counts observed in the present study were comparable to those previously reported in North African dairy systems (Tayeb et al. 2024). Such high microbial loads may reflect inadequate refrigeration, prolonged storage, or suboptimal hygienic conditions during milk production and handling. Similarly, coliform and fecal coliform counts exceeded recommended limits in several regions suggesting differences in environmental hygiene and sanitary management practices during milking, storage, or transport. Comparable findings have been reported in other Algerian dairy production systems (Ariri et al. 2023).

The higher coliform counts observed in Oued El Djemaa may reflect deficiencies in milking hygiene or equipment sanitation. In contrast, the lower fecal contamination levels observed in Zemmora could indicate relatively improved

management practices or better environmental hygiene conditions. Elevated *Staphylococcus* spp. counts in Oued Rhiou and Relizane may suggest deficiencies in udder health management or hygienic milking practices, although no direct pathogen identification was performed in the present study. Similar observations have been described in previous investigations of bovine mastitis in Algeria (Meskini et al. 2021b; Seddar-Yagoub et al. 2024).

Interrelationships between milk parameters

Spearman correlation analysis revealed strong positive association between total viable counts and both total coliforms (ρ=0.527, *P*<0.001) and fecal coliforms (ρ=0.711, *P*<0.001), while no significant relationship was observed between TVC and physicochemical parameters (Figure 1). Fat content positively correlated with minerals (ρ=0.511, *P* = 0.001), protein (ρ=0.551, *P*<0.001), lactose (ρ=0.551, *P*<0.001), and freezing point (ρ=0.339, *P* = 0.028), whereas a negative correlation was observed with pH (ρ=–0.350, *P* = 0.023). Density, solid non-fat, protein, minerals, and lactose exhibited strong intercorrelations (ρ=0.407 to 0.782, *P*<0.01) (Figure 1), reflecting coordinated variation of milk solids.

These results suggest that microbial indicators were mainly related to hygienic quality parameters, whereas physicochemical characteristics demonstrated a high degree of internal interdependence.

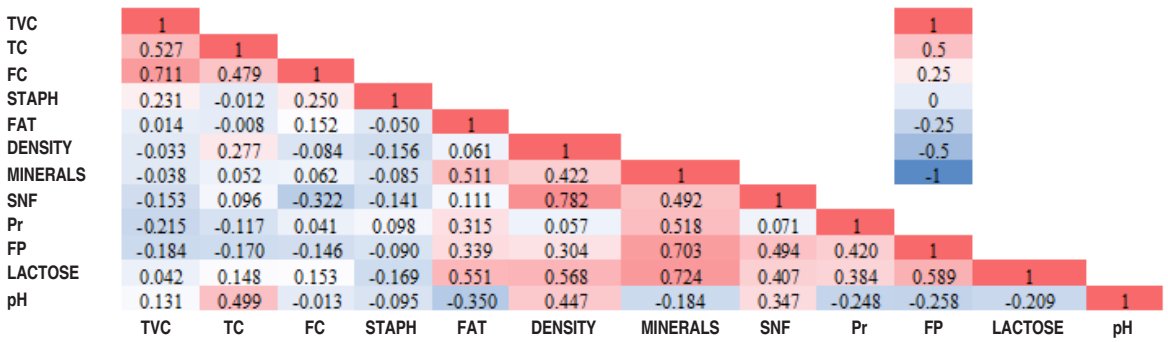


Figure 1. Associations between bacterial contamination and physicochemical characteristics of raw milk.

The positive correlations observed among TVC, total coliforms, and fecal coliforms, further support their role as complementary indicators of microbial contamination. Furthermore, the correlation between total coliforms and pH is consistent with previous studies reporting the influence of microbial activity on milk physicochemical stability (Kazeminia et al. 2023). Similarly, the positive relationship between density and SNF, protein, and lactose reflects the contribution of non-fat solids to milk specific gravity.

CONCLUSION

This study highlights marked variability in the physicochemical and microbiological quality of raw cow milk produced in smallholder dairy systems in Relizane, reflecting differences in production conditions and hygienic management practices across regions. Overall, the findings indicate that milk quality is inconsistently compliant with recommended standards, particularly with regard to compositional balance and microbial hygiene indicators. The results demonstrate that both physicochemical and microbiological attributes are sensitive indicators of production system performance and may collectively reflect underlying limitations in feeding strategies, udder health management, and milk handling practices. The observed associations between microbial indicators further support their value as complementary markers for assessing hygienic status in raw milk. These findings highlight the importance of improving milking hygiene practices, controlling environmental contamination sources, and strengthening cold-chain management within smallholder dairy production systems. The observed regional variability suggests that implementing targeted quality control strategies may help reduce inconsistencies and improve the overall quality and safety of raw milk.

Future research should focus on longitudinal monitoring programs, the integration of udder health diagnostics, including the detection of subclinical mastitis, and the assessment of antimicrobial residues to provide a more comprehensive understanding of milk safety, quality, and the sustainability of dairy production systems.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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