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Raw milk composition and sanitary quality after summer in semi-arid region

Rohmilchzusammensetzung und hygienische Qualität nach dem Sommer in einer semiariden Region

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Summary

The purpose of the present study was to evaluate physicochemical characteristics, milk coagulation properties and microbiological contents afterward the hot season to understand the impacts on raw milk quality. Fifty milk samples were collected in autumn season from the bulk tank of dairy collectors delivering milk to three major dairy industries in Setif, a semi-arid region in Algeria. Milk composition analysis showed that means of fat, solid-not-fat, protein, lactose, freezing point, total minerals, titrable acidity, density and heat stability rate were 2.66%, 8.54%, 3.18%,4.65%, –0.46°C, 0.67%, 1.89 g lactic acid/l, 1028.64 g/l and 92% respectively. Most milk samples were coagulated (78%). All physicochemical parameters were close or within the threshold values, only the point freezing was higher than the standard value. Mineral content was significantly different between dairy industries 1 and 3 ($P<0.01$), otherwise milk composition is similar in all dairy industries. Regarding microbiological content, means of total aerobic bacteria count, coagulase-positive Staphylococcus, fecal coliform and Salmonella presence were 3.87 CFU/ml, 1.36 CFU/ml, 2.74 CFU/ml and 0% respectively; however, a significant difference was observed in the microbiological contents between the dairy industries ($P<0.05$-0.01). In conclusion, physicochemical composition and sanitary quality of raw milk collected in autumn were acceptable. However, although clotting properties indicated that milk is suitable for cheese making, milk quality remains insufficient to reach requirement and demand of dairy processors of high milk quality. Further efforts are requested from the whole dairy chain to improve milk quality.

Keywords: Raw milk, Physicochemical characteristics, Microbiology, Coagulation properties, Semi-arid region.

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Introduction

Cow's milk has a main role in population diet and rural economy in most developing countries. Also, milk demand is expected to be increased by 25 percent by 2025 (FAO, 2008). In Algeria, dairy production is the second major agricultural activity contributing to the national gross domestic production after cereal products and constitutes one of the pillars of food security. However, one of the key limitations for local milk integration in the dairy chain industry is the variability of milk quality parameters at the processors level (Pistocchini et al., 2009).

Bulk tank milk analysis is now widely accepted as a useful tool for evaluating milk quality (Jayarao and Wolfgang, 2003). Microbiological contamination of raw milk takes place mainly at farm level, and bacteria may be originated from a variety of sources, ranging from inside the udder to the milking and storage equipment (Skeie et al., 2019). Nevertheless, presence of some pathogenic bacteria is a serious threat to consumer health.

In developing countries, few studies investigated milk hygienic and physicochemical composition before processing afterward the hot season to understand the impacts on milk quality. Also, no study has reported raw milk coagulation properties in North Africa, yet. Thus, the present study aims to evaluate bulk tank raw milk quality delivered to the three major dairy industries in Setif by the analysis of the main harmful bacteria to health and evaluate whether or not, after a dry and hot summer, the physicochemical characteristics and the microbiological contents, are within the standard values.

Materials and methods

The current study was conducted during autumn 2019 in three major dairy industries in Setif region, composed of two large dairy industries, one public and one cooperative, and one small private dairy industry.

Setif (North East, Algeria) is a semi-arid region, characterized by a subtropical climate with hot summers and cold to mild winters. During autumn 2019, the minimum temperature ranged from –1 to 11°C, and the maximum from 20,4 to 31,5°C. However, rainfall ranged from 0 to 43 mm (https://www. Info. Climat.fr).

Milk sampling

Fifty raw milk samples were collected in the morning from the bulk tank of dairy collectors delivering milk to three major dairy industries in Setif; samples were taken respectively from 20, 20 and 10 dairy collectors delivering milk to a public dairy industry, dairy cooperative and a small private dairy industry. The three dairy industries received daily milk from dairy collectors who collect milk from dairy producers and sell it to the industries.

For each dairy industry, the best dairy collectors in the overall amount of milk collected per season were selected for milk sampling as this reflects dairy production of several breeders in the region.

Raw milk samples were aseptically collected in sterile bottles; bottles were labeled and stored in an ice-cooled box and were transported to the laboratory within 1 to 3 hours after pick up of the first sampling at delivery sites.

Milk composition results were interpreted according to the Algerian Interministerial decree of August 18, 1993 relating to milk and dairy products, whereas those of milk microbiology were discussed according to the Interministerial decree of October 4, 2016 relating to the national microbiological criteria of foodstuffs.

FAO threshold values were considered when Algerian standard values were not indicated (Table 1).

Physicochemical Analysis

Ultrasonic Milk analyzer Lactoscan was used to determine the content of fat (% w/v), protein (% w/v), lactose (% w/v), solid-not-fat (SNF) (% w/v), freezing point (_°C) as well as salts (%). In addition, titratable acidity, expressed in g lactic acid/L, was determined after titration with NaOH in the presence of phenolphthalein (AFNOR 1993) and density (g/l) performed using lactodensitometer (AOAC 1997). However, heat milk stability was assessed after heating milk in a water bath at temperature of 100°C for 5 min and was then allowed to cool in ambient temperature. Milk was considered unstable when clots were noticed.

Milk coagulation properties

Furthermore, milk quality was evaluated for its suitability for cheese making using lactofermentation test: 5 ml of milk is incubated in tube at 37°C for 24h. After observation of milk evolution, milk was classified into one of the following four classes according to their clotting properties: liquid; flaky, digested coagulum with expulsion of a large amount of serum and coagulated, a homogeneous coagulum without serum (CTFC, 2011).

Microbiological analysis

Microbiological milk quality is evaluated by performing multiple tests including total aerobic bacteria count, coagulase-positive Staphylococcus, fecal coliforms and Salmonella. Microbiological contents were determined according to the official methods of analysis, approved by the Directorate General of Economic Control and Fraud Prevention, Ministry of Commerce, Algeria.

- **Total aerobic bacteria count (TABC):** a series of milk samples dilution were prepared, 1ml of appropriate milk dilution was transferred into sterile Petri dishes, 12 ml of sterile and pre-prepared gelose agar was pooled into the previous milk dilution that was put in the Petri dishes. After well mixing and agar solidification, petri dishes were incubated at 30 °C for 72 h and colonies were then counted.
- **Coagulase-positive staphylococcus:** milk sample was inoculated into a Giolitti and Cantoni enrichment broth. After incubation at 37 ° C for 24h, a quantity of 0.01 ml of enrichment cultures was spread on the surface of Baird-Parker medium plate. Colonies of staphylococcus which were cultured on Baird-Parker medium were subjected to the coagulase test.

TABLE 1: *Acceptability threshold values of physicochemical properties of raw milk.*

Fat (%)	Solid-non-fat (%)	Proteins (%)	Lactose (%)	Titratable acidity (g/l)	Total minerals (%)	Density (g/l)	Freezing point (°C)	Heat stability
≥ 2.8[1)]	8.5–9[2)]	3.2–3.4[3)]	3.6–5.5[2)]	1.4–1.8[1)]	<1[2)]	1030–1034[1)]	–0.53 to –0.55[2)]	Stable[1)]

[1)] Algerian Interministerial decree of August 18, 1993 relating to milk and dairy products. [2)] FAO, 1993. [3)] Muehlhoff et al., 2013.

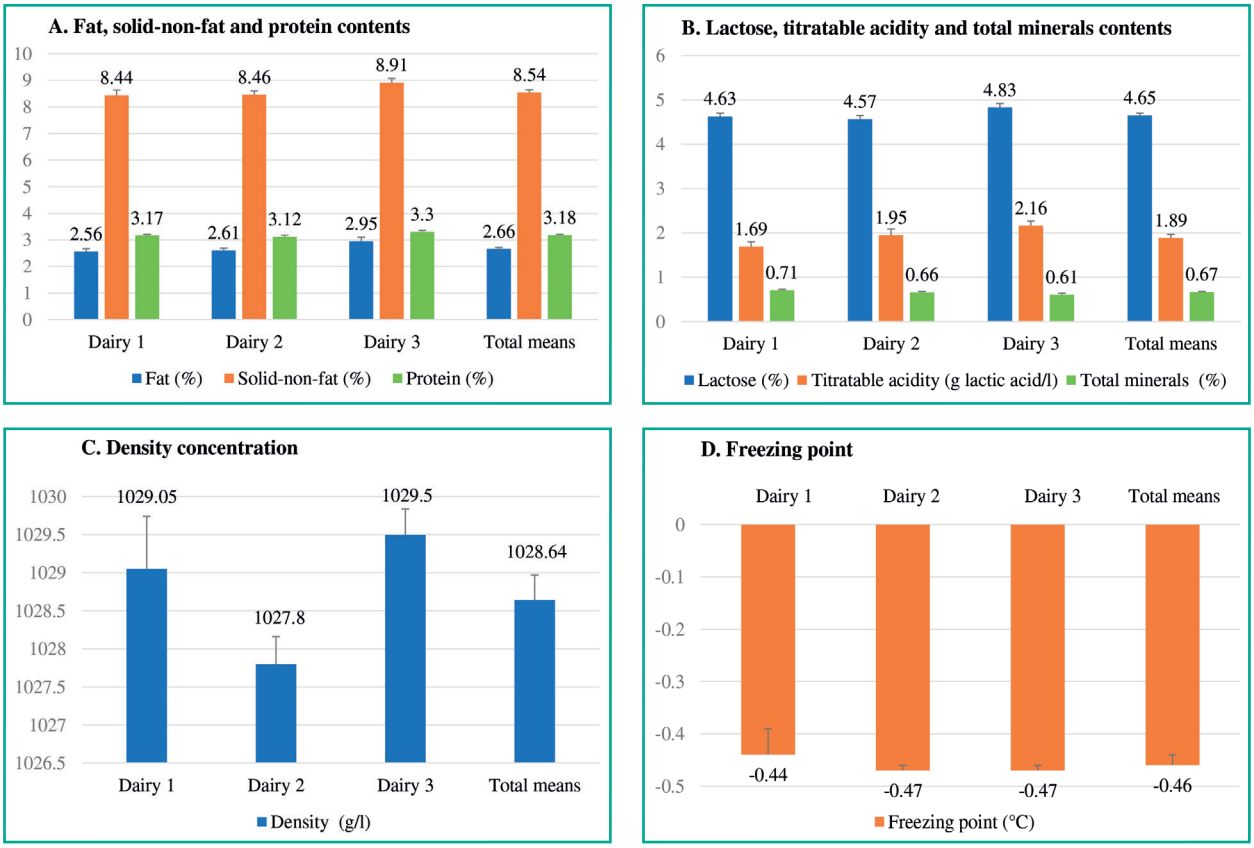

FIGURE 1A–E: *Physicochemical characteristics of raw milk in the different dairy industries.*

- **Fecal Coliforms:** after preparing a series of milk dilution, appropriate diluted milk samples were cultured in the medium of Violet Red Bile (VRB) agar and incubated at 44 °C for 24 to 48h. Red characteristic colonies were then counted.
- **Salmonella research:** detection involved four steps, pre-enrichment in buffered peptone water and incubation at 37°C for 16 to 18h, enrichment in a selective liquid medium, then enrichment cultures were streaked on Hektoen agar and incubated at 37°C for 24 h; biochemical confirmation by sub-cultivation of characteristic salmonella colonies on Triple Sugar Iron agar (TSI) and incubated at 37°C for 24 h.

For each analysis, five replications of cultures were done; colonies were then counted in each plate after consideration of the dilution factor. The average of the five replications was then calculated.

Statistical Analysis

All data were presented as mean ± SE, whereas heat stability, coagulation classes and salmonella content were presented as frequencies. Levene's test was used to control the homogeneity of variances. One-way ANOVA test was performed to check significance of differences between dairy industries followed by LSD post hoc test to compare dairy industries means. If variances were not equal, the Kruskal-Wallis test was conducted followed by the Mann-Whitney U test.

Differences in frequencies between dairy industries were assessed using Khi-square or Fisher's exact test when sample size was small. $P<0.05$ was taken as significant.

All statistical analyses were performed using the SPSS package program, version 20.0.

Results and discussion

Milk physicochemical Analysis

Results of milk physicochemical analyses are presented in Figure 1, while the acceptability threshold values are shown in Table 1.

SNF, protein, lactose and minerals contents are within the threshold value (Figure 1A and B); while, fat content was slightly below the standard set value (Figure 1A).

Evaluation of raw milk quality before processing is one of the major critical points for enhancing dairy value chain. Large dairy industries have created their self-milk control services before milk delivery which can guarantee them to receive safe and quality milk. In the case of fat content, results obtained was 2.7% which is close to that reported in Iran (2.6%) by Shojaei, and Yadollahi (2008); while, the content of protein, fat and SNF obtained in our study are far lower than those obtained in Poland and Sudan (Kuczaj 2001; Abd Elrahman et al, 2009), due to

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the low availability of forage (amount of fiber in diet), mostly replaced by concentrate during autumn season. Our findings agree with those obtained by Campos (2016), when milk constituents had lower values in drier season, period from July to December. Previous studies reported that fat and protein contents are influenced by genotype, type of breeds and feed quality (Legarto et al., 2014; Ramírez-Rivera et al., 2019).

Raw milk is material for producing fresh cream, butter and cheese; however, fat and protein contents analyzed in this study were not sufficient to satisfy dairy industries and consumer demands for producing high-quality dairy products. In fact, more than 90% of the fat and casein in milk contribute to the cheese solids (Lou and Ng-Kwai, 1992). Also, the quality and amount of cheese obtained, not only per volume of milk but also per gram of protein in cheese milk, is important for the economic outcome of the dairy industry (Wedholm et al., 2006).

Moreover, high fat content is an economic advantage for dairy collectors and producers to increase their profits because milk is priced according to the fat content by the most of dairy industries in Algeria.

In our study, milk acidity is slightly higher than the recommended standard value (Figure 1B). A similar finding was noticed by Gargouri et al., (2013) in Tunisia, where milk acidity was slightly higher than that of fresh milk; the author attributed it to the effect of agitation during milking and transport on active acidity of milk (Jandal, 1996; Czerniewicz et al., 2006). Also, poor cooling during transportation and storage can contribute to the increase in acidity which unfortunately causes rejection of milk.

Regarding milk density, which depends on protein and fats content, and SNF content were close to the standard values (Figure 1C). A value lower than 1010 g/liter would have indicated extraneous water (FAO, 1993). Apparently, density and SNF changes when milk is adultered with water or other materials are added or both misdeeds are committed (FAO, 1998).

Our result showed that freezing point mean value determined in raw milk samples during autumn were higher than the standard set value (Figure 1D), and that obtained in Netherlands in bulk tank milk samples during the winter and summer (–0.52091°C) (Slaghuis 2001). It would be explained by the differences in breed and season (Henno et al., 2008). Also, freezing point of farm tank milk is widely used to detect water adulteration of milk.

Most milk samples were heat stable (92%) as is shown in Figure 1E, which guarantees to the dairy collectors acceptance of their milk delivery by the dairy industries.

In general, all physicochemical parameters were similar between dairy industries ($P>0.05$), except for mineral content which differs significantly between the different industries ($P<0.01$), probably due to the differences in breeds, nutrition and feeding practices of breeders.

Milk Coagulation properties

Frequencies of the different classes of milk according to their clotting properties are presented in Table 2. However, most of the milk samples were well coagulated, with a frequency of 78% which is higher than the frequencies of coagulated milk noticed in France (69%) (Michel et al., 2001) and in milk samples collected from Swedish and Danish Holstein cows (61%) (Wedholm et al., 2006). To our knowledge, no study has reported milk coagulation properties in North Africa. This result states that raw milk was rich in lactic acid bacteria that will quickly take a smooth gel, so favourable for cheese making. Indeed, gelatinous type of coagulum indicates the richness of milk in flora class of technological interest (Michel et al., 2001).

TABLE 2: *Clotting properties of raw milk collected for the various dairy industries.*

Dairy industries	n (frequency) Liquid	Flaky	Digested coagulum	Coagulated	Total
1	0 (0 %)	3 (15 %)	5 (25 %)	12 (60 %)	20 (100 %)
2	0 (0 %)	1 (5 %)	2 (10 %)	17 (85 %)	20 (100 %)
3	0 (0 %)	0 (0 %)	0 (0 %)	10 (100 %)	10 (100 %)
Total	0 (0 %)	4 (8 %)	7 (14 %)	39 (78 %)	50 (100 %)

n: number of milk samples

TABLE 3: *Microbiological contents in the different dairy industries and threshold values.*

	Dairy industries	n	Means (x 10^2)	Standard Error	Threshold values[1])
Total aerobic bacteria count (CFU/ml)	1	20	6.77[a]	± 0.67	≤ 3 x 10^6
	2	20	1.81	± 0.25	
	3	10	2.20[b]	± 0.42	
	Total	50	3.87	± 0.45	
Coagulase positive Staphylococcus (CFU/ml)	1	20	1.60[a]	± 0.39	≤ 10^3
	2	20	0.59[b]	± 0.13	
	3	10	2.40[c]	± 0.57	
	Total	50	1.36	± 0.22	
Fecal coliform (CFU/ml)	1	20	4.84[a]	± 0.69	≤ 5 x 10^3
	2	20	1.36	± 0.16	
	3	10	1.31[b]	± 0.38	
	Total	50	2.74	± 0.38	
			Presence	**Absence**	
Salmonella (25 ml)	1	20	0%	100 %	Absence
	2	20	0%	100 %	
	3	10	0%	100 %	
	Total	50	0%	100 %	

n: number of milk samples. Superscript letters show significant differences between dairy industries ($P<0.05$–0.01).
[1]) Interministerial decree of October 4, 2016 relating to the national microbiological criteria of foodstuffs.

Microbiological Analysis

Interestingly, all the microbiological contents analyzed in our milk sample did not exceed the national standard values (Table 3), due probably that raw milk was exposed during transport and storage in autumn to low environmental temperatures. Indeed, if milk must be stored for one day or longer, cooling down to 4°C is necessary (FAO, 1993).

Our results disagree with those found in Cameroon by Belli et al., (2013) during autumn, contamination levels of raw milk samples varied widely in the country; about half of the samples complied with threshold values for TBC, while high coliforms counts were detected in some samples. After reviewing many studies, Metz et al., (2020) reported that most raw samples contain coliforms <100 CFU/ml in different countries, which is close that found in our study.

Low content of fecal coliform was obtained in the present study which indicates the hygiene of environment, dairy equipment, and milk good management practices (De Silva et al., 2016).

Currently, most of dairy industries choose disciplined dairy collectors who control the hygiene of the environment and the cleanliness of milk at farm level; milking and container equipment are critical for maintaining milk

sanitary quality (Ramírez-Rivera et al., 2019). In this regard, dairy collectors are forced to follow the hygiene rules imposed by the dairy industries in term of the hygiene of dairy equipment, as well as the respect of the cooling temperature in the whole milk chain from the farm to the delivery milk, otherwise milk will be rejected.

However, several studies reported raw milk samples contamination during seasons other than autumn; Elmoslemany et al. (2009) found in Egypt that TBC and coliform counts in raw milk were high in summer and low in winter, while raw milk was contaminated in spring by E.Coli in Iran (Shojaei and Yadollahi 2008), by TBC, total coliform, E. and coagulase-negative Staphylococcus in Hungary in summer (Woode et al., 2018) and by Faecal Coliforms and Staphylococcus aureus in Morocco from October 2010 to March 2011 (Hadrya et al., 2012).

However, although within threshold values in all cases, a significant difference was found in the content of TABC, fecal coliform and coagulase-positive Staphylococcus between the dairy industries ($p<0.05$-0.01), which could be explained by the different hygiene practices of dairy collectors and producers who deliver milk to the three industries.

It is very important to be aware of the danger of milk contamination on health. High prevalence of pathogenic bacteria for human health could be found in raw milk, mainly in developing countries where people can consume fresh milk (Reta et al., 2016). The safety of milk and dairy products must be ensured to protect consumers, particularly vulnerable consumers such as children.

Indeed, producing high-quality milk and dairy products that are or will be demanded by consumers can be a challenging and complex task. Governments may need to make initial investments in the dairy sector to stimulate private-sector investments (Muehlhoff et al., 2013). Nevertheless, producing high milk quality and hygienic is beneficial for costumer and economic advantage for dairy producers and industries.

Conclusion

The current study has shown that generally physicochemical composition and sanitary quality of raw milk collected in autumn collected from the dairy collectors of three major dairy industries in Setif region were acceptable. However, although clotting properties indicated that milk is suitable for cheese making, milk quality remains insufficient to reach requirement and demand of dairy processors of high milk quality for producing added-value dairy products. Further efforts are requested from the whole dairy chain, dairy, producers, collectors and industries to improve milk quality. Government should encourage investments for setting genetic breeding program and training dairy producers on cow nutritional requirements and forage production. Also, hydroponic forage production is an innovative technology that should be taken seriously in consideration in the semi-arid regions.

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Conflict of interest

The authors declare that there is no conflict of interest.

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