Research & Reviews: Journal of Agriculture and Allied Sciences

e-ISSN:2347-226X
p-ISSN:2319-9857

The Effect of Storage Temperature and Duration on the Composition and Bacteriological Quality of Raw Milk

Naceur M'hamdi[1]*, Rachid Bouraoui[2], Cyrine Darej[1], Abir Mahjoub[2], Lamia Hassayoune[3], Hajer M'hamdi[4] and Latifa Lanouar[5]

[1]Laboratory of Animal and Food Resources, National Agronomic Institute of Tunisia, Tunisia
[2]Higher School of Agriculture of Mateur, University of Carthage, Tunisia
[3]Ministery of Agriculture, OEP Mahdia, Tunisia
[4]Ministery of Agriculture, CRDA Ben Arous, Tunis, Tunisia
[5]Higher Institute of Agronomy of Chott-Meriem, Sousse, Tunisia

Research Article

Received date: 08/05/2018
Accepted date: 23/05/2018
Published date: 31/05/2018

***For Correspondence**

Naceur M'hamdi, Assistant Professor, Laboratory of Animal and Food Resources, National Agronomic Institute of Tunisia, 43 Avenue Charles Nicolle, Tunis 1082, Tunisia.

Tel: +216-71-287-110
Fax: +216-71-892 785
E-mail: naceur_mhamdi@yahoo.fr

Keywords: Bacteriological quality, Milk, Storage condition

ABSTRACT

Storage of milk production in cold temperatures is a current Tunisian legislation request. However, there is no specification of a limit period for this. The main aim of this study was to assess the effect cold storage and during transport on physicochemical and bacteriological quality of milk in the region of Mahdia. An investigation was carried out on 61 dairy farms in which milk was stored for 2, 24 and 48 hours and transported under refrigeration until the center. Moreover, 61 milk samples were analyzed for bacteriological quality, chemical composition and physical parameters. Results showed that at a temperature less than 5°C the average values of TBC and CT were 7.9 10^5 and 2.06 10^4 cfu/ml, respectively and that TBC increases from a temperature ranging from 5 and 7°C to reach an average of 9 10^5 cfu/ml and from a higher temperature (26°C) the TBC and CT were 3.7 10^6 and 3.74 10^4 cfu/ml, respectively. Analysis of variance showed that the physical characteristics (acidity and pH), were significantly affected by length and temperature of storage ($P<0.05$). The mean value at 26°C is 6.69. For a temperature between 5 and 7°C, pH is 6.7, while for a temperature less than 4°C, the pH is 6.73 and acidity is 15.64 ± 1.04°D. On the other hand, at 26°C, acidity is 15.74 ± 0.73°D. However, no significant effect was noticed of the cold storage and transport on chemical parameters of milk (Fat, protein, lactose and total solid). The mean levels of fat and protein content, lactose and EST are respectively 31.46, 30.69, 49.39 and 114.2 g/l.

Finally, we conclude deterioration in bacteriological quality of the milk after transport.

INTRODUCTION

The demand for dairy products is increasing due to population growth in most developing countries. The government of each country aims to set up several high capacity dairy units to increase milk processing. The challenge is that, how to provide safety raw milk any time for the dairy unit for processing. Sometimes, dairy farms are located far from the cities and the dairy processor ought to set up collection center to stored milk for some hours before it reaches processing unit. In practice, it has been observed that after the implementation of granelizada milk collection, there is storage for more than 48 h at the source of production because the expansion tanks allow milk storage of the various milking, thereby reducing transportation costs [1]. Elrahman et al.

e-ISSN:2347-226X
p-ISSN:2319-9857

[2] reported that rapid cooling is recommended on farm, it could help the farmer to sale the safety product. In Tunisia, the most problem is the seasonality of milk production and the higher temperature during the peak, which makes its management difficult. Many studies have been carried out to highlight how is important to store milk cool at optimal temperature [3] to have product with excellent quality and avoid contamination before processing [4] and good storing conditions of dairy products [5].

The aim of this work was to investigate how quality and composition of raw milk changes during cold storage and transport.

MATERIALS AND METHODS

Farms were eligible to participate in the study if they shipped milk to a common dairy processor, had milk quality data (bacterial counts and SCC) determined for most milk loads produced. The experiment was carried out from February to June in 61 dairy farms of Mahdia at the center of Tunisia. Farms were visited monthly, to assess changes in management practices. Temperature was recorded for milk loads either at arrival to collection point (for farms that stored milk in tankers or at pickup (for farms that had a bulk tank).

Milk sampling

Raw milk samples (n=61) were collected using sterile utensils from all selected farms, from stored milk production and with a minimum of three samples per farm. During sample collection, the storage time of milk at each specific farm was recorded 2 h just after milking, 24 h storage, and 48 h storage. A total of 61 samples were randomly collected of which 26 samples were collected from refrigerated tanks installed on farms, 25 from non-refrigerated tanks, 7 from Tanks for transporting milk on arrival at the collection center and 3 samples from refrigerated tank at the collection center. Milk samples were collected with 2, 24 and 48 h of storage, which were characterized as mixed refrigerated raw milk. The samples were collected in dry clean sterile glass bottles (25 ml), preserved in ice box at ≤ 4°C and transported to the laboratory of centrale laitière "Vitalait" for microbiological examination.

Milk constituents and physical characteristics

The milk constituents (Fat, Protein, Lactose, and TS) and physical characteristics (Density and Freezing Point), of the milk samples were determined by milk analyzer using milk analyzer Lactoscan 90. Acidity was measured by titration with Dornic solution (0.1111 N NaOH), using phenolphthalein as the indicator according to AOAC [6]. The pH of milk samples was measured using a pH meter.

Microbiological analysis

The microbiological analysis was carried out at the Laboratory of Vitalait Mahdia, Tunisia. Samples of raw milk were analyzed for enumeration of Total Bacterial Count (TBC), Coliforms Count (CC).

Statistical analysis

The statistical analysis was performed using Statistical Analysis Systems (SAS Institute Inc., Cary, NC; 2002-2008, Release 9.2). General Linear Models were used for the determination of the effect of location and marketing channel on the microbiological quality of raw milk. Means were separated by LSD test at P ≤ 0.05. Descriptive statistics including average, standard deviation, and variability coefficient, minimum and maximum was done.

RESULTS AND DISCUSSIONS

Descriptive statistics

Table 1 summarizes the physico-chemical parameters of milk at the farm level. The average milk density at 20°C is 1028.96 ± 0.83 with minimum and maximum values of 1027 and 1030 respectively. These values correspond to the norms cited by Goursaud [7] and Vignola [8], which vary between 1028-1036 and 1028-1035 and Labioui et al. [9] who mentioned a density measured at 20°C between 1028 and 1033. However, the average values of the density recorded in our study are above the threshold of the standard of acceptance of raw milk by INNORPI which is of the order of 1.028 (NT 14.41, 2007). Fat Content (FC) is 31.93 ± 3.5 g/l and ranges from 22.86 to 38.34 g/l. This mean value is significantly lower than that reported by Bousselmi et al. [10], which ranged from 35 to 45 g/l for whole milk. However, it is higher than the Tunisian standard (30 g/l). Mean protein content (PC) was 30.47 ± 2.33 g/l with values ranging from 26.9 to 37.07 g/l. Although this average level is above the acceptance threshold for milk in Tunisia (28 g/l), the mean of PC must be between 30 and 35 g/l. The mean value of lactose is 49.03 ± 2.64 g/l. It is in the normal range for raw milk, i.e., 40 to 50 g/l [9]. The mean Total Dry Extract (TDE) is 114.4 ± 2.46 g/l with a minimum of 108.65 g/l and a maximum of 119.5 g/l. For the mean freezing point, it is estimated to be -0.516, it varies from -0.490 to -0.540°C. According to Amiot et al. [11], when the freezing point is above -0.530°C, wetting may be suspected. Regarding acidity, its mean value is 15.68 ± 0.87°D with a minimum of 14 and can go up to 17°D. The average value of titratable acidity agrees with those of [12] which varies between 15 and 18°D. The means of pH vary from 6.58 to 6.79 with an average of 6.72 ± 0.06. Since the pH of a fresh milk must be between 6.6 and 6.8 according to Tunisian standards 14,141 (2007).

e-ISSN:2347-226X
p-ISSN:2319-9857

Table 1. Mean acidity, pH, Freezing point and chemical composition values of raw milk.

Parameters	N	Means	Std	Minimum	Maximum
Density 20°C	61	1028.96	0.83	1027	1030
FP	61	-0.516	0.01	-0.540	-0.490
Acidity (°D)	61	15.68	0.87	14	17
pH	61	6.72	0.06	6.58	6.79
FC (g/l)	61	31.93	3.5	22.86	38.34
Protein (g/l)	61	30.47	2.33	26.9	37.07
Lactose	61	49.03	2.64	34.5	52.14
TDE	61	114.4	2.46	108.65	119.5

TDE=Total Dry Extract, FP=Freezen Point, FC= Fat Content

Characteristics of milk bacterial counts

The results of the microbiological analyzes of the milk taken upstream of the production chain **(Table 2)** show a higher contamination. The average TBC is 2.2 10^6 cfu/ml, ranging from 13 10^4 to 14 10^6 cfu/ml. TBC increases during collection to an average of 2.9 10^6 cfu/ml. The TBC in this study is lower than that reported by Srairi et al. [13] and El Labioui et al. [9] who reported an average TBC of 42.4 and 6.34 10^6 cfu/ml, respectively. However, it is equal to 1.84 10^6 cfu/ml found by Grillet et al. [14] at the farm level, which subsequently increased rapidly at the collection centers. In the same way, Titouche et al. [15] reported a change in the microbial density of 5.3 10^6 cfu/ml at the farm level to 6.4 10^6 cfu/ml at the collection center level. This can be explained by Swai and Schoonman [16], by an intense microbial multiplication, favored by the lack of hygiene practices during milking and cold storage on the farm, the use of plastic buckets for and the distance between the farm and the collection center.

The mean value of coliform count (CC) is 2.9 10^4 cfu/mL, ranging from 0.06 10^4 to 15 10^4 cfu/ml just after milking. In peddlers, this value becomes 5.6 10^4 cfu/ml. However, contamination at the collection center tanks became more remarkable with an average CT of 9.5 10^4 cfu/ml **(Table 2)**. The coliform contents found are lower than those mentioned by Sraïri et al. [13] which are 4.1 10^5 cfu/ml. However, they exceed the rate recorded by Labioui et al. [9] which is 5.2 10^3 cfu/ml. The poor hygiene of the herd, contaminated water, unhygienic milking practices and poorly maintained equipment can lead to a higher number of coliforms in raw milk [17]. Also, the higher count of bacteria may be due to bad cleaning system and bad handling from farms to collected center).

Table 2. Microbiological milk quality at different point of sampling

	Variable	N	Mean	SD	Minimum	Maximum
Farms	TBC (10^6 cfu/ml)	61	2.2	1.7	0.13	14
	CC (10^4 cfu/ml)	61	2.9	2	0.06	15
Transport	TBC (10^6 cfu/ml)	7	2.9	2.2	0.8	6.3
	CC (10^4 cfu/ml)	7	5.6	4.9	0.97	16
Collection point	TBC (10^6 cfu/ml)	3	2.6	0.6	1.9	3.1
	CC (10^4 cfu/ml)	3	9.5	4.6	4.7	14

TBC= Total Bacterial Count, CC=Coliforms Count

e-ISSN:2347-226X
p-ISSN:2319-9857

Effect of storage period and temperature on total bacterial counts

The variation of TBC is slightly influenced by milk temperature. Thus, the number of germs in raw milk varies little according to temperature. At a temperature below 4°C, the mean of TBC is 7.9 10^5 cfu/ml. The TBC level increases from a temperature between 5 and 7°C to an average of 9 10^5 cfu/mL and from a higher temperature (26°C) the TBC becomes 3.7 10^6 cfu/ml. Our results for chilled milk are significantly higher than 0.75 10^5 cfu/ml obtained by Maldaner et al. [18]. In the same context, Guimarães [19] found poor milk quality at temperatures below 4°C, ranging from 1 10^6 to 1.68 10^7 cfu/ml. At room temperature (36.9°C on average) Labioui et al. [9] reported an average TBC of 6.38 10^6 cfu/ml greater than the value found in this study. The microbial growth is due to the maximum growth of the mesophilic flora at average temperatures of 25 to 30°C.

The variation of TBC according to the age of the milk is not significant (P>0.05). Since the milk stay time does not exceed 2 hours without cold on the farm. Milk at room temperature has highest TBC value (3.7 10^6 cfu/ml). Storage at farm level during 24 and 48 h at a temperature below 4°C, allows us to obtain milk with mean values of TBC, respectively 8.4 and 7.5 10^5 cfu/ml **(Table 3)**.

The temperature of raw milk (age <2 h) was higher in the first hours of storage, because at the time of sampling, the milk was at room temperature 22°C. Kanyeka [20] reported that the level of milk contamination after two hours of milking at room temperature is very high (4.89 10^6 cfu/ml). Our results agree with those of O'Connell et al. [21] who considered that the addition of raw milk to the refrigeration tank (T<4°C) on the farm, at each milking. Throughout the storage period (48 h), limited the rate of deterioration of milk quality. So, they recorded an average TBC for 24 h. $Log_{10}3$, 44 and $Log_{10}3$, 47 cfu/ml after 48 h. From these results, we can conclude the importance of cold on the farm. Indeed, European Standard 853 (2004) recommends that immediately after milking, milk should be kept in a clean, designed and equipped place to avoid contamination. It should be cooled immediately at no more than 8°C in the case of daily collection or not more than 6°C if the collection is not daily. According to Fagundes et al. [22], at the second hour after milking, the temperature should be 4°C.

Effect of storage period and temperature on coliforms counts

The variation of CC as a function of temperature is significant (P<0.05). However, many coliform bacteria can grow at low temperatures **(Table 3)**, resulting in an average of 2.06 10^4 cfu/ml at a temperature less than 5°C, which represents a notable variation compared to uncooled milk (3.74 10^4 cfu/ml). Our results are superior to those reported by Maldaner et al. [18] at 4°C (0.6 10^2 cfu/ml), and similar to those of Labioui et al. [9] at room temperature (2 10^4 cfu/ml).

The change in CC slightly depends on the age of the milk. Indeed, moderate variability was observed for total coliforms with an average value of 3.74 10^4 cfu/ml for milk stored for two hours. For milk stored on the farm, a remarkable difference was envisaged in storage for 24 hours and 48 hours with averages of 1.3 and 3 10^4 cfu/ml, respectively. This difference is explained by the hygiene conditions and practices in stockbreeders whose samples have been stored for 24 hours. Our results come to an agreement with Veisseyre [23] who reported that contaminated milk (containing more than 2 10^5 cfu/ml) can only be stored for 24 hours, even at temperatures below 4°C. It can be concluded from these results that even favorable conditions for the storage of milk on certain farms (refrigeration). Up to its delivery can in no way mask general hygiene practices which are very disappointing, especially during milking (Washing hands and teats. washing utensils. filtering milk) and a very good indicator in the procedure of cleaning milking facilities.

Table 3. Effect of storage period and temperature on bacteriological quality of milk

Temperature	Storage duration (hours)	N	Parameters	Means	Min	Max
0 - 5°C	24	10	TBC (× 10^6 ufc/ml)	0.77	0.13	1.8
			CC (× 10^4 ufc/ml)	1.3	0.06	6.7
0 - 5°C	48	14	TBC (× 10^6 ufc/ml)	0.78	0.16	2
			CC (× 10^4 ufc/ml)	3.2	0.2	15
5 - 7°C	48	2	TBC (× 10^6 ufc/ml)	0.91	0.23	1.6
			CC (× 10^4 ufc/ml)	3.4	1.8	5
(26°C ≤)	2	25	TBC (× 10^6 ufc/ml)	3.7	0.36	14
			CC (× 10^4 ufc/ml)	3.74	0.16	12

TBC= Total Bacterial Count, Coliforms Count

e-ISSN:2347-226X
p-ISSN:2319-9857

Effect of storage time and temperature on milk pH and acidity

The average raw milk pH in this study was 6.69 at 26°C in the range of pH found by different authors in tropical conditions [3,13]. When milk was stored at room temperature, milk pH decreased immediately after 24 hours **(Table 4)**. In contrast, when milk was stored in the refrigerator at 4°C, we found very insignificant variation between milk pH from 24 to 48 hours of storage time. When milk is stored in the refrigerator at 4°C, the variation of milk pH is very slow. Analysis of variance (ANOVA) showed that acidity and pH are significantly influenced by storage time and temperature ($P<0.05$). The pH measured under the various conditions is stable and normal (pH=6.69 at 26°C). When the temperature varies from 5 to 7°C, the average pH value is 6.7. For a temperature not exceeding 4°C, the pH takes an average value of 6.73. In terms of acidity, it is noted that it is proportional to temperature. Indeed, the acidity increases with the increase of temperature. At a temperature below 4°C, the average acidity is 15.64 ± 1.04°D. At 26°C, the average acidity is 15.74 ± 0.73°D. It should be noted that for 4% of samples at a temperature of 7°C, the acidity is 15.5°D **(Table 4)**. The value of acidity in our study is consistent with the mean value found by Labioui [9] at room temperature (16.75°D at 36.9°C).

Table 4 shows that milk stored during 24 and 48 h has an average acidity of 15.4 ± 0.73 and 15.8 ± 1.14°D and an average pH of 6.74 ± 0.04 and 6.72 ± 1.14, respectively. But milk stored only for 2 h at ambient temperature has an average acidity of 15.74 ± 0.73°D and a pH of 6.69 ± 0.07. When the milk was stored on the farm at 4°C, no significantly variation was found between 24 and 48 hours of storage.

Table 4. Effect of storage time and temperature on Milk pH and acidity.

Temperature	Storage time (hours)	N	Parameters	Means	Min	Max
0 - 5°C	24	10	Acidity	15.4	14	16.5
			pH	6.74	6.64	6.79
0 - 4°C	48	14	Acidity	15.82	14	18
			pH	6.72	6.66	6.79
5 - 7°C	48	2	Acidity	15.5	15.5	15.5
			pH	6.7	6.68	6.72
26°C	2	25	Acidity	15.74	14.5	17.5
			pH	6.69	6.58	6.99

Our results agree with those of Millogo et al. [24] who noticed that when the milk is stored at 4°C. The pH variation is very slow (6.5 during 24 h to 6.4 during 48 h). Likewise, for acidity, Da Silva et al. [1] recorded a value of 15 for 24 h and 16 at 48 h. According to Karim et al. [17] in a brief time after milking, acidity increases substantially due to bacterial activity. The degree of bacterial contamination and the temperature at which milk is stored are the main factors influencing acid formation. Therefore, the level of acid depends on the cleanliness of the production and the temperature at which the milk is stored. Da Silva et al. [1] stated that the storage of refrigerated milk in the bulk tank maintained at temperatures <7°C up to 48 h does not improve milk quality because of changes in milk composition.

CONCLUSION

Refrigerated storage of raw milk is a prerequisite in dairy industry. Few researchers have examined the effect of storage conditions on milk stored in bulk tanks located on farms. Our study is a better reflection of conditions experienced on commercial farms. In this study, milk entering the tank was of bad microbial quality, which may be a critical factor influencing the results. However, temperature abused conditions in the farming and processing environments can significantly affect the microbiological quality of raw milk. In conclusion, storage temperature and time can affect microbiological quality of raw milk. The number of bacteria remaining in raw milk reaching the processing factory from the small-hold farmers shows a positive trend with its milk holding time in transportation.

REFERENCES

1. Da Silva MAP, et al. Effect of temperature of storage on the composition and microbiological quality of raw milk. Afr J Microbiol Res. 2015;9:1480-1486.

2. Abd Elrahman SMA, et al. Effect of storage temperature on the microbiological and physicochemical properties of pasteur-

e-ISSN:2347-226X
p-ISSN:2319-9857

ized milk. Food Sci Technol. 2013;14:115-121.

3. Sboui A, et al. Comparaison de la composition physicochimique du lait camelin et bovin du Sud tunisien ; variation du pHet de l'acidité à différentes températures. Afrique Sci. 2009;5:293-304.
4. Forsbäck L, et al. Day-to-day variation in milk yield and milk composition at the udder-quarter level. J Dairy Sci. 2010;93:3569-3577.
5. Faye B and Loiseau G. Sources of contamination in dairy supply chains and approaches to quality control. In: Food safety management in developing countries: Proceedings of the international workshop, CIRAD-FAO, 11-13 December 2000, Montpellier (France). 2002;p:5.
6. AOAC. Official methods of analysis (15th Edn). Association of Official Analytical Chemists, Washington DC, USA, 1990.
7. Goursaud J. Coagulation enzymatique du lait. In: Biotechnologie (vol 1), 1985.
8. Vignola CL. Science et technologie du lait: transformation du lait. Presses internationales polytechnique, Montréal, Canada. 2002;p:600.
9. Labioui H, et al. Étude physicochimique et microbiologique de laits crus. Bull Soc Pharm Bordeaux. 2009;148:7-16.
10. Bousselmi K, et al. Facteurs de variation des taux de matière grasse et protéique du lait de vache de race Holstein en Tunisie. Renc Rech Ruminants 2010;17:399.
11. Amiot J, et al. Composition, propriétés physicochimiques, valeur nutritive, qualité technologique et techniques d'analyse du lait. In: Science et technologie du lait: transformation du lait (Vignola CL Edn). Presses internationales polytechnique, Montréal, Canada. 2002;pp:1-54.
12. Carole V. Sciences et technologie du lait, transformation du lait. Fondation de technologue laitière. St Lourent Montréal. 2002;p:600.
13. Sraïri MT, et al. Effect of cattle management practices on raw milk quality on farms operating in two-stage dairy chain. Trop Anim Health Prod. 2009;41:259-272.
14. Grillet N, et al. Qualité sanitaire du lait cru tout au long de la filière dans le district de Mbarara et la ville de Kampala en Ouganda. Revue Élev Méd vét Pays trop. 2005;58:245-255.
15. Titouche Y, et al. Assessment of microbiological quality of raw milk produced at Tizi Ouzou area (Algeria). Asian J Anim Vet Adv. 2016;11:854-860.
16. Swai ES and Schoonman L. Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. Asian Pac J Trop Biomed. 2011;1:217-222.
17. Karim MH, et al. Study on physicochemical and microbial quality of available raw, pasteurized and UHT milk during preservation. Int J Sci Inv Today. 2013;2:150-157.
18. Maldaner N, et al. Evaluation of microbiological quality of raw milk produced at two properties in the far west of Santa Catarina, Brasil. Food Public Health. 2012;2:79-84.
19. Guimarrães CPA. Impacto da assistência técnica sobre a qualidade do leite, Goiânia. Dissertação (Me-strado em Medicina Veterinária) - Escola de Veterinária, Universidade Federal de Goiás. 2008;p:82.
20. Kanyeka HB. Assessment of microbial quality of raw cow's milk and antimicrobial susceptibility of selected milk-borne bacteria in Kilosa and Mvomro districts, Tanzania (MSc thesis). University of Agriculture, Morogoro, Tanzania. 2014;pp:50-59.
21. O'Connell A, et al. The effect of storage temperature and duration on the microbial quality of bulk tank milk. J Dairy Sci. 2016;99:3367-3374.
22. Fagundes CM, et al. Presença de Pseudomonas spp em função de diferentes etapas da ordenha com distintos manejos higiênicos e no leite refrigerado. Cienc Rural. 2006;36:290-293.
23. Veisseyre R. Technologie du lait: constitution, recolte, traitement et transformation du lait (3rd Edn). La Maison Rustique, Paris. 1975;714:104-106.
24. Millogo V, et al. Effect of storage time and temperature on raw milk composition of dairy cattle in tropical conditions. Afr J Dairy Farm Milk Prod. 2014;2:104-108.