

Microbiological and Chemicophysical Comparative Study of Cow's Milk in the Commune of Mograne (Gharb region, Morocco)

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Abstract

With the aim of studying the bacteriological quality of the milk of the town of mograne in comparison with a study made in 2007; ten samples of the cow's milk restarted in two series were analyzed by physico-chemical and microbiological point of view. The obtained results, show that analyzed milk present successful physico-chemical characters parrapport in 2007. So the temperature of milk after draft has little meadows 37°C and the pH is 6.68. The bacteriological load in FMAT is of average of 5.67.10⁵ ufc / ml and the average contents total and faecal coliformes are respectively 9,07.10³ ufc / ml and 4,53.10³ ufc / ml. The realized analyses also mark the total absence of staphylococci, salmonellas and clostridium; a good indicator on the sanitary state of cows treated as well as on the quality of analyzed milk. These results are better than those registered in 2007, what informs about the impact of the practices of hygiene and breeding on the bacteriological quality of the milk.

Keywords: Bacteriological quality, Physicochemical quality, Milk, practices of hygiene, Mograne commune

Introduction

Milk production is a strategic sector of the Moroccan agricultural policy, particularly for its roles as provider of animal protein facing rapid population growth, creation of jobs and wealth. The policy of milk production is managed by the Moroccan government with a management plan in order to contribute its *growth* and *strength* (Dairy Plan, 1975) [1].

In fact, Morocco is one of the leading of milk importing countries and others milk products to satisfy the high demand for these vital commodities in spite of efforts deployed to reform the sector by adopting a second plan (2000-2020) [2] which compensates the deficiencies of the first one. Major deficiencies of milk production in Morocco are the result of climatic conditions, technical and socio-economic constraints that hinder the development of the cattle industry and the milk production in the country.

Therefore, development of milk production sector requires first to highlight these barriers to be able to raise production. This may be possible by the implementation of a multidimensional approach taking into account potentialities of exploitation, levels of economic growth and the socio-economic situation of farmers [3]. So, we have to identify strategies of producers to offer shares of development which are compatible with the producers capacity and production systems dynamics [4].

In this labor we are interested to identify production issue and milk quality by studying physicochemical and microbiological characteristics of milk in Mograne commune before and after the establishment of an accompaniment program, [5] in order to highlight the impact of livestock and hygienic practices on production and milk quality.

Materials and methods

Selection of samples

The sampling of milk to be examined is selected from farmers, collectors milk and also from mixing milk that has been collected in the center. So, we randomly selected milk for analysis from a collector, three farmers who arrived first at collection center and finally a sample from the first tank of the mixture milk in the center. These samples represent the first batch. The second series includes the same sampling, after three days, for other breeders and collector, and a collection of the second tank.

The samples were collected in sterile glass bottles and are immediately transported to the laboratory in a cooler. They are submitted to physicochemical and microbiological analyzes a few minutes after their arrival. Samples were held in May 2008, from 7am.

Physicochemical - Analysis

The temperature of the milk of the center is almost fitting the ambient temperature. The temperature of the milk in tanks is always about 4°C which sufficient to keep the milk fresh until its delivery by the truck from the factory.

The PH measurement is done by a pH-meter type WTW meter Inolab 522 fitted with a combined electrode and

previously calibrated by the buffer solutions at pH=4 and pH=7.

The acidity of the milk is calculated by titration of the sample of milk by a solution of sodium hydroxide (NAOH), 0.1N in the presence of a colored indicator (phenolphthalein to 1 %). The acidity was expressed in "degree Donric °D (1°D = 0, 1g of lactic acid/liter). For 10ml of the test sample, we add a drop of phenolphthalein at 1%.

The acidity is titrated using soda N/9 content in a in a Mohr burette with a system of dipping We stir constantly until the color changes to pale pink in time of 10 seconds.

Measuring the density of the milk is used for studying the wetting of milk. It varies according to the species (Alias, 1984). However the density of the milk cow is between 1,030 and 1, 035.

For practicing, we homogenized the sample of milk, pour it into a measuring cylinder of 500 ml and plunge it the thermo-lacto-densimeter at 20 degrees, after we wait for the stability. The reading of the density value is done at the top edge depending of temperature.

If the temperature is different temperature of 20°C, it is bringing to 20°C according to the following formula:

$$DC=DL \pm 0.2 \Delta T$$

DC: density Corrected, **DL :** density read **ΔT:** difference in temperature between the milk and 20°C.

The fat content is determined by the method acido-butyrométrique de Gerber [2] which consists in an treatment of the milk by the sulfuric acid and separation by centrifugation in the presence of iso-Amyl alcohol of the fat released (Afnor, 2001).

The determination of the dry matter content is done after drying milk at a temperature of $103 \pm 2^\circ\text{C}$, for 3 hours, until evaporation of the water and stabilisation of the weight of the milk to dry. (we takes 10ml of milk for each sample).

The determination of the ash content of the milk, is done by incineration of the dry matter at 530°C until constant weight (Afnor, 1993).

The chosen method for the detection of milk wetting is to calculate the dry extract of milk, using the following formula (Hammama, 1996): $M = (90 - \text{ESD}) / 90$

Microbiological - Analysis

Milk is a product rich in nutrients and water. It represents a favorable culture media of a large number of microorganisms. The microbiological analyzes are carried out for the purpose to find:

- The indicators of the hygienic quality of milk which are:
 - The enumeration of total aerobic mesophilic flora (FMAT) is counted on PCA agar (Plate Count Agar) incubated for 24 h at 30°C.
 - The search for total and fecal coliforms: They are sought on gelose desoxycolate citrate agar (DCL) incubated 24 hours at 37°C for total coliforms and at 44°C for fecal coliforms.
- The germ of industrial interest:
 - An enumeration of lactic acid bacteria responsible for the fermentation and the acidification of milk; they are counted on the MRS agar (Man Rogosa Sharpe, Difco, Detroit, United States) and incubated 48 hours at 30°C.
 - The yeasts and molds are counted on the media potato dextrose agar (PDA) and incubated for 48 hours at 30°C.
- The harmful bacteria (pathogens) :
 - Fecal streptococci are counted on the sodium azide after incubation 48 hours at 37°C.
 - The staphylococci are counted on the media Chapman (or Mannitol Salt Agar) containing a high concentration in NaCl (75 %) tolerable only by the staphylococci, the incubation is done at 37°C, during 24 to 48 hours.
 - For salmonella, we carried out a pre-enrichment on the media selenite cysteine 12 hours at 37°C, followed by an enrichment on broth on the tetra thionate during 24 hours at 37°C, and then the count and isolation has been made on the media SS (Salmonella Shigella) after incubation during 24 hours at 37°C.
 - Les clostridiums sulfitoréducteurs are counted on the growing media Reinforced Clostridium Agar tubes in order to promote the anaerobic conditions, with a heat treatment for 10 min at 80°C to activate the spores of Clostridium which results in: they can persist under latent form in the milk, germinate when conditions are favorable and secrete toxic substances. The tubes are incubated 48 h at 37°C. Only the black colonies are counted.

Microbiological analysis of milk is carried out in three stages: the preparation of dilutions, seeding on the growing media and enumeration of microorganisms.

Result and discussion

Physicochemical analysis

Physicochemical analysis of different samples (Table 1) show that the temperatures of milk are between 36 and

37°C, so close to the ambient temperature, meaning that cows are kept *healthy* and well fed. PH measurement of raw milk recorded an average value of 6.68. Similar results have been developed by [9] and [10]. The pH of our milk is therefore located within the range of standards that is between 6.5 and 6.7 [11].

The values of the acidity of the samples is 16.20°D. This value is lower than 2007 samples which was 16.95°D [12]. However, This value is placed in a norm of an interval of 15 and 18 °D [13].

The values of the density at 20°C shows an average of 1.030. This value is within the normal range between which is between 1.028 and 1.035 [11] This demonstrates that the milk in our study contains good proportions of fat and dry matter.

The average of fat content measured was 31.6 g/l, a value higher than that found by us in 2007. The amounts of fat obtained in our case are located in the range of 28.5 to 32.5 g/l, which are accredited by AFNOR [6].

The values of the solids content obtained are about 120.59 g/l, slightly greater than the average measured by us in 2007 which was in the order of 119.95 g / l [12]. These values are lower than that obtained by [9] which was 128g/l but higher than that found by [14] (117.5 g/l). These differences are explained by the diet and also by the variability of cattle breed.

Microbiological analysis

The results of microbiological analyzes of different samples studied after the monitoring program show that the average load of *total aerobic mesophilic flora* was $5.67.10^5$ CFU/ ml. It's lower than that the results obtained by us in 2007 (ml $5.7.10^6$ CFU/ml) and also to that found by [15] which is 2.10^7 CFU/ml and by [14] which is $7.4 10^6$ CF /ml .

The average content of total and fecal coliforms are respectively $9,07.10^3$ and $4,53.10^3$ CFU/ml which are less than those values obtained in 2007 which are $2,15.10^4$ CFU/ml and $4,45.10^3$ CFU/ml. These values are lower than those provided by [15] ($1,8.10^5$ CFU/ml of fecal coliforms) and [14] ($5,2.10^3$ CFU/ml and for fecal coliforms and $2,0.10^4$ CFU/ml for total coliforms).

The average value of streptococci measured was approximately $6,46.10^2$ CFU/ml, this value is lower than obtained in 2007 ($1,12.10^3$ CFU/ml) [12]. This content is also lower than that announced by [16] which is $18,3.10^3$ CFU/ ml. But apparently higher than found by [14] which is $0,4.10^3$ CFU/ml. It's can be explained by contamination of milk during milking or by influence ambient temperature.

The analyzes mark the total absence of Staphylococci, Salmonella and Clostridia which suggests that the health of treated cows is good and the quality of milk is acceptable according hygienic point of view.

The average load of lactic acid bacteria in our milk is from $9,59,10^5$ CFU/ml, higher than found in 2007 which was $7,5.10^5$ CFU/ml. The result obtained is slightly higher than that found in [14] which is of $8,3.10^5$ CFU/ml.

The load is of yeasts found is $9,71.10^3$ CFU/ml, this value is lower than that found by us in 2007 ($1,21.10^4$) CFU/ml [12] and that obtained by [14] which is $1,22.10^4$ CFU/ml. Thus, the result obtained in this study is a normal content that will allow the necessary fermentation for the production of dairy derivatives.

Conclusion

Several studies on the quality of milk were made in the region. The microbiological quality of milk produced in this region has been undertaken before (Reviews in Biology and Biotechnology 2008. BioAlliance Canada-Morocco), also another study on the classification of raw milk from different regions of Morocco was performed. For the same reason, the assessment of the nutritional quality of milk necessary to highlight the variability in the nutritional quality of milk production, evaluate the quality of milk produced in those regions and determine the influencing factors, to improve production and profitability.

Milk quality plays an important role in marketing and quantity of milk produced plays a very important role in meeting market demand in terms of milk and its derivatives.

Of the samples studied, the fat content, fat solids and milk protein cooperatives have much variety in the way of the farm cooperative. The principal component analysis was used to explore the variability between the two series, pastoralists and the effects of factors that may affect the content of the constituents of milk density, fat and dry matter.

It appears from this physicochemical study that the raw milk analyzed in 2008 showed improved values to those found in 2007. This improvement concerning physicochemical characteristics is due to close monitoring program in the field of animal husbandry and hygiene which farmers have benefited from these practices. Therefore, microbiological characteristics mark very satisfactory results. This is confirmed by the low load source of fecal contamination which clearly indicates the very positive impact of hygiene practices introduce during milking and the maintenance of the dairy herd concerning the microbial quality of milk.

REFERENCES

AFNOR, (2001). Lait - Détermination de la teneur en matière grasse - Méthode gravimétrique (méthode de

- référence). NF EN ISO 1211, décembre 2001, 21 p.
- AFNOR, (1993). *Contrôle de la qualité des produits alimentaires : lait et produits laitiers : analyses physicochimiques*. Paris La Défense : AFNOR, 1993, 4^e éd., 581 p.
- Alais (C.), (1984). La micelle de caséine et la coagulation du lait. In *Science du lait : Principes des techniques laitières*. Paris : Ed. Sepaic, 1984, 4^e ed., 723-764.
- Araba A., Benjelloun S., Hamama A., Hamimaz R. et Zahar M. (2001). Organisation de la filière laitière au Maroc. Option Méditerranéennes. Série. B / n° 32 : 52-53.
- Bayoumi, (1990). Studies on composition and rennet coagulation of camel milk. Kieler Michw essenschaft for Schungsberichte, 42:3-8.
- F. GUESSOUS (1991). *Production fourragère et systèmes animaux*. Actes Editions, Institut Agronomique et Vétérinaire Hassan II, Rabat, Maroc, (1991) 128 p.
- Gaursaud.j, (1985). Composition et propriétés physicochimiques. In Luquet, FM. Lait et produits laitiers. Lavoisier, Paris, 1985, tome 1.
- GUERRA L, (2008). Contribution à la connaissance des systèmes d'élevage bovin dans la région semi aride de Sétif, Algerie universite farhat abbas setif memoire Pour l'obtention du diplôme d'ingenieur d'etat en sciences agronomiques.94p
- Hammama A., (2002). Hygiène et prophylaxie dans les étables laitières. Cours de formation des techniciens de l'Office Régional de Mise en Valeur Agricole l'Haouz.Mai 2002, Marrakech.
- Hammama (A.), El Mouktafi (M.), (1990). Étude de la qualité hygiénique du lait cru produit au Maroc. - *Maghreb Vét.*, 5, 17-20.
- Hammama A., (1996). Hygiène du lait à la production.- Rabat : Proceedings de la journées sur la qualité du lait organisée par la direction de l'élevage, Institut Agronomique et Vétérinaire Hassan II et l'association nationale des éleveurs de bovins.
- Labioui, (H.) Elmoualdi (L.), Benzakour (A), El Yachioui (M.), Berny (H),Ouhssine (M.), (2009). Etude physicochimique et microbiologique de laits crus. *Bull. Soc. Pharm. Bordeaux*, 148, 7-16
- Mennane. Z, (2008). Lait et produits laits entre la tradition et la biotechnologie. Etude physicochimique et microbiologique. Thèse doctorat 2008 en Microbiologie. Université Ibn Tofaïl Faculté des sciences kénitra.175p
- MADANI T., HUBERT B., LASSEUR J., GUERIN G., (2001). Association des bovins, des ovins et des caprins dans les élevages de la suberaie algérienne. *Cahiers d'études et de recherches francophones / Agricultures*, 10 (1). Pp : 9-18.
- RHIAT Mohammed, Mohammed OUHSSINE, Youness CHBAB, Mahjoub AOUANE, (2010). Pratiques d'élevage et d'hygiène sur la production laitière bovine dans un centre de collecte du Gharb, Maroc. *Bull. Soc. Pharm. Bordeaux*, 149, 17-32 pages
- RHIAT Mohammed, LABIOUI Hicham, Youness CHBAB, Mahjoub AOUANE Mohammed OUHSSINE (2013). Caractérisation physicochimique et microbiologique du lait bovin et evaluation de l'activite bactericide des bacteries lactiques. *Tunisian Journal of Medicinal Plants and Natural Products TJMPNP "*.Vol 10(2), 1-12.

Table 1: Physicochemical analysis of samples of raw milk in May, 2008

Sample	Temperature of milk after milking (°C)	pH	Acidity D°	Density	Fat content (g/l)	Solid content (g/l)
1	6,69	16,7	1,030	33,0	121,30
2	37	6,68	16,6	1,032	32,5	121,50
3	36,8	6,71	15,6	1,029	31,5	120,65
4	36,9	6,69	16,3	1,028	29,5	119,16
5	37,1	6,68	15,8	1,031	31,0	119,45
6	36,9	6,72	16,1	1,028	32,5	121,15
7	36,9	6,67	16,3	1,028	29,5	119,66
8	37	6,69	15,9	1,031	31,5	119,05
9	36,9	6,71	16,8	1,030	32,0	120,81
10	36,8	6,7	16,7	1,029	29,5	119,22
Average	36,9	6,69	16,24	1,0296	31,3	120,20

- The sample No. 1 is the mixture of milk collected bins collection center
- Sample No. 2 represents the collector of milk
- samples of 3 to 10 represent milk farmers

Table 2. Microbiological analysis samples of raw milk in May, 2008

Sample	Total aerobic mesophilic flora	Coliforms		Staphylo-cocci	Salmo-nella	Clostridium	Strepto-cocci	Lactic bacteria	Yeast
		Total	Fecal						
1	5,410 ⁵	11.10 ³	7,2.10 ³	0	0	0	7,2.10 ²	9,3.10 ⁵	13.10 ³
2	9,7.10 ⁵	18.10 ³	9,1.10 ³	0	0	0	11.10 ²	7,9.10 ⁵	12.10 ³
3	2,7.10 ⁵	2,6.10 ³	0,9.10 ³	0	0	0	5,910 ²	9,9.10 ⁵	4,8.10 ³
4	9,2.10 ⁵	3,7.10 ³	2,4.10 ³	0	0	0	*	6.10 ⁵	6,5.10 ³
5	3,2.10 ⁵	8,7.10 ³	1,8.10 ³	0	0	0	3, 2.10 ²	21.10 ⁵	9,3.10 ³
6	4,6.10 ⁵	13.10 ³	6,3.10 ³	0	0	0	6,7.10 ²	8,7.10 ⁵	11.10 ³
7	11.10 ⁵	21.10 ³	8,8.10 ³	0	0	0	8,3.10 ³	9.8.10 ⁵	14.10 ³
8	2,2.10 ⁵	5,3.10 ³	3,9.10 ³	0	0	0	*	12.10 ⁵	7,8.10 ³
9	5,9.10 ⁵	3,3.10 ³	5,9.10 ³	0	0	0	5,2.10 ²	8,3.10 ⁵	9,5.10 ³
10	2,8.10 ⁵	4,1.10 ³	4,1.10 ³	0	0	0	4,2.10 ²	11.10 ⁵	9,2.10 ³
Average	5.67.10 ⁵	9,07.10 ³	4.53.10 ³	0	0	0	6,46.10 ²	9,59.10 ⁵	9,71.10 ³

Table 3. Microbiological analysis samples of raw milk (2007, before the program and 2008, after the program)

	2007(before the program)	2008 (after the program)
Total aerobic mesophilic flora	5,7.10 ⁶	5.67.10 ⁵
Coliforms	Total	2,15.10 ⁴
	Fecal	4,45.10 ³
Staphylococci	0	0
salmonella	0	0
Clostridium	0	0
Streptococci	1,12.10 ³	9,59.10 ⁵
Lactic bacteria	7,5.10 ⁵	9,59.10 ⁵
Yeast	1,1.10 ⁴	9,71.10 ³