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Evaluation of the Hygienic Quality of Raw Cow's Milk in Oujda City Morocco

K. Mchiouer[1], S. Bennani[1], N. Sh. El-Gendy[2] and M. Meziane[1]

[1]Department of Biology, Faculty of Sciences, Laboratory of Water Sciences, Environment and Ecology, University Mohammed I, Oujda, Morocco.
[2]Petroleum Biotechnology Laboratory, Department of Process Development, Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt.

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The aim of this study is to determine the microbiological quality of raw cows' milk of Oujda city. Raw milk samples are collected randomly between June 2014 and May 2015 from 20 Mahlaba (dairies) for microbiological evaluation. The samples are analyzed to determine total mesophilic aerobic bacteria (TMAB), total coliform, fecal coliform, *staphylococcus aureus,* fecal streptococci, proteolytic bacteria and lactic acid bacteria. The results of bacterial count showed that there is a variation between all the milk samples and a period effect is also observed. The mean counts of total mesophilic aerobic bacteria from all sale points are between 1.76×10^6 and 40.17×10^6 CFU/ml. Milk samples reveled counts total coliform and fecal coliform ranging from 0.58×10^5 to 11.10×10^5 CFU/ml and from 0.60×10^3 to 14.64×10^3 CFU/ml, respectively. *Staphylococcus aureus* are also detected in all samples with counts ranging from 0.35×10^3 to 3.08×10^3 CFU/ml. Fecal streptococci are found in all milk samples, at counts ranging from 0.16×10^2 to 2.18×10^2 CFU/ml. And finally, proteolytic and lactic acid bacteria are between 0.3×10^3 and 2.86×10^3 CFU/ml and 2.37×10^6 and 24.14×10^6 CFU/ml respectively. These results indicate a lack of compliance with good manufacturing practice at milking, collection and transportation of raw milk.

Keywords: Raw milk, Hygiene, Microbiological quality, Pathogens, Oujda, Morocco.

Milk is well known as a medium that favors the growth of several microorganisms, especially bacterial pathogens (Nada *et al*., 2012). Generally, milk from healthy cows is sterile inside the mammary gland. Whereas, number and type of bacteria that might occur in milk immediately after milking is associated with direct contact with contaminating sources in a dairy farm environment (Angulo *et al*., 2009). High temperatures also promote the growth of pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella*, *Listeria monocytogenes*, *Escherichia coli*, and *Clostridia* (Mellenberger and Kirk, 2001).

However, keeping milk immediately after milking process in clean containers at refrigerated temperatures may retard the increase of initial microbial load and prevent the multiplication of microorganisms in milk between milking at the farm and transportation to the processing plant (Chye *et al*., 2004; Millogo *et al*., 2010). In this context, the assessment of the bacteriological quality of milk is necessary and essential to identify the points of failure in order to protect consumers and improve its hygienic appearance.

In Morocco, cows' milk production has a particular status in the agricultural development programs. Indeed milk production has been

* To whom all correspondence should be addressed.
E-mail: nourepri@yahoo.com

intensively promoted since the seventies due to significant demand for milk due to a population that has become increasingly important (Sraïri *et al*., 2005; Afif *et al*., 2008; Mchiouer *et al*., 2016). Many investigations have been performed on the quality of raw milk at different levels of the dairy sector in Morocco ; Ounine *et al*. (2004) and Taybi *et al*. (2014) evaluated the bacteriological quality of raw milk produced in the region of Gharb, while, Afif *et al*. (2008) performed it in the region of Tadla. To our knowledge, no comprehensive study on the hygienic quality of milk produced in the Eastern region of Morocco has been undertaken previously. Thus, the aim of this study is to determine the microbiological quality of raw cows' milk within the direct sale points (Mahlaba) in Oujda area.

MATERIALS AND METHODS

Milk samples collection

Raw cows' milk samples are randomly collected from 20 Mahlaba (dairies) across Oujda city, Morocco. Samples are collected between June 2014 and May 2015. The collection is done in the morning under possible aseptic conditions; approximately 100 - 300 ml milk are sampled from containers from each individual dairy into a sterile labeled bottle. Samples are delivered to the laboratory in a cool box (< 4°C) and tested immediately upon arrival.

Microbiological analysis

Each sample of milk is subjected to the following tests after preparing dilutions:

- The total mesophilic bacterial count, was realized by pipetting 1ml of each sample dilutions (10^{-1} to 10^{-6}) into a Petri dish and mixed well with 15 ml of sterile standard plate count agar (PCA). The plates were then incubated at 30°C for 48 hours (Ghazi *et al*., 2010).
- Coliforms were performed on MacConkey medium, the separation of fecal coliform and total coliform were based on the incubation temperature which was 37°C for 24 hours for the enumeration of total coliform and is 44°C for 24 hours for the enumeration of fecal coliform.
- *Staphylococcus aureus* were enumerated on the medium Mannitol Salt Agar (Chapman, 1945), where, the plates were cultivated for 24 hours in an aerobic atmosphere at 37°C. Then the appeared golden yellow or orange colonies were counted.
- The enumeration of fecal streptococci was done using KF *Streptococcus* Agar as a selective medium after inoculating the plates, they were incubated inverted at 37°C for 46 to 48 hours (Kenner *et al*., 1961).
- Bacterial proteolytic activity was determined

Table 1. Statistical characteristics of total mesophilic aerobic bacteria (TMAB), total coliform and fecal coliform for raw milk based on the time (month).

Month	Total mesophilic aerobic bacteria				Total coliform				Fecal coliform			
	Mean ($\times 10^6$)	SD	CV	F	Mean ($\times 10^5$)	SD	CV	F	Mean ($\times 10^3$)	SD	CV	F
June-14	17,13 ^{cd}	8,91	52,01	28,10	7,42 ^{abc}	4,83	65,09	6,62	1,150 ^{ef}	1,176	102,26	7,83
July-14	31,21 ^{b}	13,49	43,23		5,19 ^{cde}	4,36	84,07		10,25 ^{b}	6,40	62,42	
Aug.-14	40,17 ^{a}	11,79	29,35		11,10 ^{a}	5,98	53,85		5,39 ^{cd}	3,85	71,38	
Sept.-14	21,80 ^{c}	7,82	35,86		2,831 ^{def}	1,64	57,75		2,512 ^{def}	1,191	47,40	
Oct.-14	9,40 ^{ef}	2,02	21,55		8,90 ^{abc}	5,75	64,77		14,64 ^{a}	7,45	50,89	
Nov.-14	4,23 ^{fg}	2,57	60,67		10,84 ^{a}	5,40	49,86		7,78 ^{bc}	6,47	83,25	
Dec.-14	1,76 ^{g}	1,22	69,42		0,588 ^{f}	0,651	110,85		4,94 ^{cde}	4,76	96,44	
Jan.-15	3,97 ^{fg}	1,09	47,95		1,563 ^{ef}	1,235	79,05		2,625 ^{def}	1,652	62,95	
Feb.-15	7,38 ^{efg}	1,72	23,32		10,90 ^{a}	3,66	33,60		4,80 ^{cde}	3,72	77,43	
Mar.-15	3,92 ^{fg}	1,38	35,19		6,30 ^{bcd}	5,10	81,03		0,60 f	0,338	56,34	
Apr.-15	11,90 ^{de}	1,82	15,34		9,82 ^{ab}	7,33	74,63		5,25 ^{cde}	3,18	60,60	
May-15	10,81 ^{de}	2,05	19,03		1,025 ^{ef}	1,032	100,68		1,545 ^{def}	1,458	94,40	

Mean values within columns marked with different letters differ significantly at P Â 0.05
SD: standard deviation
CV: Coefficient of variation
F: Ficher value

Table 2. Statistical characteristics of *staphylococcus aureus*, fecal *streptococci*, and fecal *coliform*, proteolytic bacteria and Lactic acid bacteria for raw milk based on the time (month)

Month	*staphylococcus aureus*				Fecal *streptococccci*				proteolytic bacteria				Lactic acid bacteria			
	Mean ($\times10^3$)	SD	CV	F	Mean ($\times10^2$)	SD	CV	F	Mean ($\times10^3$)	SD	CV	F	Mean ($\times10^6$)	SD	CV	F
June-14	1,025 ^{de}	0,937	91,33	7,09	1,375^{bc}	0,794	57,76	5,79	1,638^{bcd}	0,778	47,52	7,33	14,48 ^{b}	6,95	48,02	10,67
July-14	0,375 ^{e}	0,212	56,57		0,675^{cde}	0,570	84,46		1,450^{bcd}	1,311	90,45		16,55^{b}	7,35	44,39	
Aug.-14	3,088 ^{a}	0,960	31,09		2,125^{ab}	1,394	65,58		2,863^{a}	0,840	29,34		24,14^{a}	7,89	32,69	
Sept.-14	2,086 ^{bc}	1,575	75,50		0,162^{e}	0,091	56,38		0,637^{ef}	0,311	48,85		12,80^{b}	9,50	74,23	
Oct.-14	1,587 ^{cd}	1,246	78,49		1,162^{bcd}	1,263	108,61		2,196^{ab}	0,840	38,25		6,30^{cd}	2,75	43,76	
Nov.-14	2,575^{ab}	1,112	43,18		2,100^{ab}	1,814	86,39		1,388^{cde}	0,549	39,56		22,61^{a}	6,73	29,77	
Dec.-14	0,350^{e}	0,177	50,65		0,363^{de}	0,320	88,40		1,075^{def}	0,738	68,67		14,86^{b}	6,55	44,08	
Jan.-15	1,388^{cd}	1,198	86,35		1,675^{b}	0,315	18,81		1,925^{bc}	0,592	30,76		2,921^{d}	2,09	71,57	
Feb.-15	1,363^{cd}	0,868	63,74		2,813^{a}	0,837	29,77		0,425^{f}	0,320	75,20		15,16^{b}	4,21	27,80	
Mar.-15	0,400^{e}	0,226	56,69		1,263^{bcd}	0,691	54,70		2,150^{abc}	1,317	61,25		10,85^{bc}	4,38	40,39	
Apr.-15	1,225^{cde}	0,433	35,38		0,425^{cde}	0,381	89,60		2,038^{bc}	0,986	48,37		2,375^{d}	1,14	48,31	
May-15	1,725^{bcd}	0,680	39,41		1,950^{ab}	1,414	72,52		0,350^{f}	0,244	69,99		14,34^{b}	4,45	31,07	

Mean values within columns marked with different letters differ significantly at P Â 0.05
SD: standard deviation
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by agar diffusion assay using skim milk agar (10% skim milk powder, 0.5% peptone, 1.5% agar) incubated at 39°C for 48 hours, where the occurrence of clear zones around the colonies is indicative of proteolysis (Stulova *et al*., 2010).

- Lactic acid bacteria were isolated on MRS agar (De MAN *et al*., 1960) after incubation at 30°C for 72 hours.

Statistical analyses

The analyses were performed using Minitab Software. All values were presented as means ± standard deviations. Statistical differences between groups of samples were determined by one-way ANOVA. Relative proportions were compared using Fisher's exact test and a probability value of less than 0.05 was defined statistically significant (Pyz-£ukasik *et al*., 2015).

RESULTS AND DISCUSSION

The mean of all bacteriological parameters studied based on time (month) are shown in Table 1 and Table 2.

Total mesophilic aerobic bacteria (TMAB) generally inform us on the hygienic quality of raw milk (Beerens *et al*., 2000). Mean TMAB in analyzed milk were ranged between 3.92×10^6 and 40.17×10^6 CFU/ml, the differences in bacterial contamination observed between months are statistically significant. Indeed, bacterial contamination in the summer period was much greater than the contamination in the winter period. Adjlane-Kaouche *et al*. (2014) results also showed that the average germ count is clearly higher in the hot season. The deterioration of the raw milk quality in many dairies (Mahlaba) might be due to the lack of good production practices including the mix between the stored evening milk with the morning new one (Afif *et al*., 2008). Milk samples revealed counts total coliform and fecal coliform ranged between 0.58×10^5 and 11.10×10^5 CFU/ml and between 0.60×10^3 and 14.64×10^3 CFU/ml, respectively. In Morocco, the number of coliforms carried in raw milk is usually high. The study carried by Ounine *et al*. (2004) in Gharb region showed values of 1.07×10^7 CFU/ml and 1.99×10^6 CFU/ml for total coliforms and fecal coliforms, respectively. Afif *et al*. (2008) results averaged; 6.31×10^5 CFU/ml and 12.17×10^3 CFU/ml for total coliforms and fecal coliforms, respectively in the region of Tadla. Coliforms bacteria loads are also reported to be significantly high in summer than in winter in the study of Lues *et al*. (2010). The presence of coliforms and pathogenic flora in milk is probably originated from cows' udder and milking utensils (Kivaria *et al*., 2006). *Staphylococcus aureus* are also detected in all samples with counts rangined between 0.35×10^3 and 3.08×10^3 CFU/ml. This value is lower to that found in other regions of Morocco, that was reported by Taybi *et al*. (2014); 2.15×10^4 CFU/ml and also the one reported by Ounine *et al*. (2004); 5.37×10^4 CFU/ml. The differences observed between months were also statistically significant in fact the higher average was observed during the month of August. *Staphylococcus aureus* are a particular indicator of the presence of the subclinical mastitis in the dairy cattle they have the ability to be transmitted from animals to humans (Adesiyun *et al*., 1998). Microbial contamination in raw milk depends also on the temperature at which it is stored and the time that elapses between milking and collection (Cempirkova, 2006). Fecal *streptococci* are found in all milk samples, at counts ranged between 0.16×10^2 and 2.18×10^2 CFU/ml. The presence on fecal *streptococci* reflects a problem of environment contamination (Ghazi *et al*., 2010). Finally, proteolytic and lactic acid bacteria were between 0.3×10^3 and 2.86×10^3 CFU/ml and 2.37×10^6 and 24.14×10^6 CFU/ml, respectively. The differences observed between months were also statistically significant. Spontaneous acidification of different tested milk is linked to the relative proportions of technological interest flora and milk spoilage flora which are the proteolytic bacteria. Lactic acid bacteria produce lactic acid and they are used for technological transformations of milk (Nawaz *et al*., 2011; Mchiouer *et al*., 2017). High temperature (> 8°C) favors the growth of lactic acid bacteria, especially if it is associated with unfavorable conditions of transportation (Pistocchini *et al*., 2009).

CONCLUSION

The results of this study clearly indicate that microbiological quality of raw milk sold in the Mahlaba of Oujda city, Morocco, is poor. The presence of pathogens and high microbial counts affect the quality of raw milk as well as its derivatives products. Pathogenic bacteria in

raw milk concerns public health, since drinking milk is still considered good for health especially in rural population. The safety of raw cow milk is influenced by a combination of management and control measures along the entire dairy supply chain. Control of animal health and adherence to good milking practices are important in reducing the microbial load in raw milk.

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