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Hygienic Quality of Raw Cow's Milk Feeding from Domestic Waste in Two Regions in Morocco

Z. MENNANE[1], M. OUHSSINE, K. KHEDID† AND M. ELYACHIOUI
College of Sciences, Laboratory of Microbial Biotechnology, Department of Biology, University Ibn Toufail, BP 133 Kénitra, Morocco
†*National of Healthy Institute, Avenue Ibn Battouta Rabat Agdal, BP 769*
[1]Corresponding author's e-mail: zakaria92t@yahoo.fr

ABSTRACT

Thirty six random samples of raw milk from cows feeding from domestics waste were collected from two regions in Morocco. Samples were analyzed for microbiological and physico-chemical properties. Microbiological properties included Total Plate Count (TPC), Total coliforms (TC), Fecal coliforms (FC), Enterococci (SF), *Staphylococcus aureus*, Salmonella, Lactic acid bacteria (LAB) spore forming bacteria (SFB) and Yeast. The results showed higher counts for all the micro organisms studied. Averages of TPC, TC, FC, SF, *S. aureus*, LAB, SFB and Yeast were $9.98x10^6$, $5.93x10^4$, $1.56x10^4$, $2.87x10^5$, $1.2x10^6$, $1.29x10^6$, $7x10^4$ and $9.63x10^5$ cfu/mL, respectively. Physico-chemical properties were as follows: pH 6.63, titrable acidity: 20.1°D, conductivity: 3.54 ms. The microbial profiles found had non-conformance to the Standard. Based on the exceedingly high microbial counts found in this study, it could be concluded that this milk type poses a serious health risk in the study areas.

Key Words: Raw milk; Waste; Hygienic; Micro organisms; Health

INTRODUCTION

Milk is a major component in human diet all over the world, but it also serves as a good medium of the growth of many micro organisms, especially pathogenic bacteria. Thus, the quality control of milk is considered essential to the health and welfare of a community. Also, all cases of dairy illness continued to be of bacterial origin, pathogens that have involved in communicable diseases associated with the consumption of milk include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter*, *Yersinia* pathogenic *Escherichia coli* and *Clostridium botulinum* (Adesiyun *et al*., 1995; Hahn, 1996; Graaf *et al*., 1997).

To ensure microbiological safety of raw milk, Moroccan regulation (MAMVA, 2003) require some conditions. However, the lack of basic infrastructure and funds affect the rate of microbial growth and spoils milk very fast (Collins *et al*., 1995). Adding at this problem, the socio-economic situation of some citizen have led at informal farmers having cowshed and utilizing the domestic waste for feeding their cows, coupled with un-hygienic practices in the milking process, lacking of refrigeration facilities. This emerging problem has been amplified by ignorance amongst the traditionally disadvantages community regarding the necessity of milk pasteurization. Most of such milk is produced and consumed locally in city by large number of people. Such milk could transmit tuberculosis, brucellosis and salmonellosis. The epidemiological impact of such diseases could be considerable.

Several studies were carried out in many regions in Morocco to evaluate the quality of raw cow milk (Hamama *et al*., 1989; Zineddine *et al*., 1996; Amhouri *et al*., 1998), but there is no investigation concerning this type of milk. Thus the present study was conducted to evaluate the microbiological quality, to investigate the chemical characteristics of milk available at producers' level in some cowshed in Kenitra and Sale province. Also, the comparison with normal bovine milk was carried out. It constitutes a preliminary study in order to present a first report.

MATERIALS AND METHODS

Physicochemical analysis. pH and conductivity: were measured by using pH–meter apparatus (Crison Micro pH, 2000).

- Titrable acidity: was determined as described by the International Dairy Federation (IDF, 1989).

Microbiological Analysis

Standard plate count (SPC). Appropriate dilution (10^{-1} up to 10^{-6}) of the samples was plated on Standard Plate Count Agar (PCA, Biokar, France). The plates were incubated at 30°C for 48 h.

Coliforms counts. Coliforms were enumerated on Desoxycholate Lactose Agar (Difco, USA). The plates were incubated at 37°C for total colifoms and at 44°C for faecal coliforms for 24 h. Isolated colonies were cultured on Trypticase Soy Agar (TSA, Oxoid) and incubated at 37°C for 24 h for further identification. The purified cultures were

stored at 4°C until identification.

Enumeration of *Staphylococcus aureus* was performed on Mannitol Salt Agar (Chapman) (Difco, USA). The plates were incubated at 37°C for 48 h. Yellow colonies were counted and checked for gram and catalase reactions. Also, the isolated were checked for their coagulase on rabbit plasma. Catalase positive Gram negative colonies were spread cultured on Trypticase Soya Agar slants for further characterization.

Enterococci. The MPN (most probable number) using 3 tubes per dilution (10^{-2} to 10^{-4}) were determined on Azide Dextrose Broth (Difco, USA). Incubation was done at 37°C for 24 h. Tubes that had shown growth were propagated on Ethyl Violet Azide broth (Difco USA) and incubated at 37°C for 24 h. Positive tubes were revealed by growth and formation of a violet precipitation in the bottom of the tubes. The number of positive tubes is reported to the Mac Grady table for the most probable number of enterococci in the sample.

Salmonella. Detection of Salmonella was carried out according to the International Standard Organization protocol (ISO0, 1990). Thus, 25 mL of the sample were added to 100 mL of sterile buffered peptone water (BPW) and incubated for 18 h at 37°C. 2 tubes of tetrathionate broth and 2 tubes of selenite cystein broth (Merck, Germany) were inoculated with 1 mL from the BPW and incubated for 24 h at 37°C. Positve tubes of both media were streaked on Hektoen agar (Merck, Germany) and typical Salmonella colonies blue green white with or without dark center, were confirmed using API 20 E test Kit (Biomerieux, France). Or method described by Poelma and Silliker (1984) was used for the identification of the suspectd colonies blue green white with or without dark center.

Spore forming bacteria. The initial dilution was exposed to 80°C for 10 min to destroy vegetative cells, then 2, 1 and 0.5 mL of this heat actived (dilution) were transferred to SPS Agar in tubes and incubate at 30°C for 24 h. Dark colonies were counted.

Yeast. The enumeration of yeast was done in potato Dextrose Agar (PDA, Merk), plates were incubated at 30°C for 3 days. Total yeast count was carried out using Potato Dextrose Agar (PDA, Merck) the plates were incubated at 30°C for 3 days.

Lactic acid bacteria (LAB). Enumeration of Lactic acid bacteria was determined using MRS medium incubated at 30°C for 48 h. After incubation, colonies were enumerated, recorded as colony forming units (cfu) per milliliter of the products.

RESULTS AND DISCUSSION

Physico-chemical and microbiological analysis according to the cattle sheds. Population bordering on refuse tip. use the waste for feeding their cattle in particular the milch cows. In this work 36 Milk samples taken from cow feeding by domestic waste from tow regions Kenitra and sale in Morocco have been analysed.

Table I. Physicochemical analyses by cattle shed

Cattle shed	Parameters	pH	Acidity (°D)	Condictivity (ms)
1	6	6,7	21,5	3,5
2	12	6.55	21.65	4.5
3	6	6.66	21.66	3.44
4	6	6.62	15.5	2.74
Total	36	6.63	20.1	3.54

The Table I shows that all samples analysed had a pH between 6.55 and 6.7 with an average of 6.63 and a high titrable acidity (20.1 D°). However samples have a weak conductivity varing from 2.74 to 4.5 ms and a average of 3.54. The pH results were similar to that reported by (Hamama, 1989; Amhouri *et al*., 1998) and to the result of cow's milk with normal food. The higher value of acidity of milk samples makes of them inappropriate for pasteurization (risk of coagulation with heating).

Hygiene quality was determined by the enumeration of SPS, total coliforms, faecal coliforms and Enterococci. The result (Table II) indicated high contamination of milk samples: SPS ($6.5x10^4$ cfu/mL to $2.17x10^7$ cfu/mL & an average $1x10^7$ cfu/mL, TC: $3.6x10^3$ cfu to $15.25x10^4$ cfu/mL with an average of $5.93x10^4$ cfu/mL, FC: $3.3x10^3$ cfu to $2.8x10^4$ cfu/mL with an average $1.56x10^4$ cfu/mL, SF: $1.5x10^4$ cfu/mL to $6.08x10^5$ cfu/mL with an average $2.87x10^5$ cfu/mL germs/mL) (Table II).

This higher contamination was probably originated from cow's udder. The rates of S. aureus found in the examined milk samples are very variable "$1.26x10^5$ to 4.3 x $l0^6$ germs/mL with an average of $1.2x10^6$ *S. aureus*/mL. This result is higher than results found by Hamama (1989); Fook *et al.* (2004) and the cows milk with normal food (witness). These values are sufficiently higher for the production of entérotoxines staphylococcic. The contamination of the milk by S. aureus is often original but can also occur after handling draft in non-hygienic conditions.

Physico-chemical and microbiological analysis according to the seasons. The 36 samples were followed according to four seasons the result obtained were summurized in (Table III & IV). The milk examined have an average values of pH vary from 6.54 in summer to 6.71 in winters and a high acidity of 18 D° in autumn to 22.81 D° in summer. However the conductivity is weak vary from 2.79 in autumn to 4.17 in summer.

Although the pH values of milk samples analysed are similar to those found by Hamama (1989); Amhouri *et al*. (1998) and the milk witness, the acidity of our samples still higher compared to their values.

The enumeration of SPS according to different seasons indicates an important contamination varies from $6.4x10^4$ cfu/mL in springs to $3x10^7$ cfu/mL in the autumn is probably the result of intense microbial multiplication. This contamination is a result of augmentation of wastes in the summer and autumn.

Table II. Microbiological analyses by cattle shed

Cattle shed	Numbers of samples	SPC (10^4 cfu/mL)	T C (10^4 cfu/mL)	F C (10^4 cfu/mL)	Staph (10^4 cfu/mL)	S F (10^4 cfu/mL)	SFB (10^4 cfu/mL)	Salm (10^4 cfu/mL)	lactic Bacteri (10^4 cfu/mL	Yeasts (10^4 cfu/mL)
1	6	6,5	15,25	2.8	38	23,8	11	0	16.6	30,8.
2	6	178	0.45	0	0	*	*	0	31	252
3	12	2177	0.36	0	12.6	1.5	3	0	47.35	69
4	6	1633	7.67	0.33	433	60.8	*	0	424	33.7
Total	36	998	5.93	1.56	120	28.7	7	0	129	96.3
witness	6	1000	0	0	*	1.23	0	*	2.64	8.6

Table III. Physicochemical analyses per season

Season	Nbre of samples	pH	Acidity (°D)	Condic (ms)
Winter	6	6,71	21,49	3,51
spring	6	6.6	21	4.7
f summer	12	6.54	22.81	4.17
autumn	12	6.68	18	2.79
Total	36	6.62	20.6	3.68

Table IV. Microbiological analyses per season

Season	Numbers of samples	SPC (10^4 cfu/mL)	T C (10^4 cfu/mL)	F C (10^4 cfu/mL)	Staph (10^4 cfu/mL)	SF (10^4 cfu/mL)	Salm (10^4 cfu/mL)	SFB (10^4 cfu/mL)	lactic Bacteri (10^4 cfu/mL)	Yeasts (10^4 cfu/mL)
winter	6	6,5	15,25	2.8	38	23,8	0	11	16.6	30,8
spring	6	6.4	0.1	*	0	*	0	*	62	4.3
summer	12	177	0.56	0.15	2.5	0.75	*	*	0.87	256
autumn	12	3000	4	0.165	226	30.4	0	1.5	258	75.85
Total	36	795	5	1.03	66.62	18.31	0	6.25	84.36	91.8
witness	6	1000	0	0	*	1.23	0	*	2.64	8.6

Bact lact: lactic bacterium; TC: total coliforms; fecal coliforms; SPS: Standard Plate Count; SF: streptocoques fecal; SFB: spore forming bacteria heat-resisting; Staph: *Sthaphylococcus aureus*; salmo: salmonella; condic: average conductivity.

The milk is strongly contaminated by total coliforms, faecal coliforms and Enterococci. The result (Table IV): TC ($1x10^3$ cfu/mL in spring to $15.25x10^4$ cfu/mL), FC ($1.5x10^3$ cfu/mL in summer to $2.8x10^4$ cfu/mL in winter), SF is also considerable especially in season of autumn ($3.04x10^5$ cfu/mL).

The presence of an important flora of fecal origine in autumn season in believed milk indicates a draft (contamination of the udder) but also a certain contamination by the hands of drover of cow feeding from domestic waste.

The rates of S. aureus found in deferent seasons are very variable "$2.5x10^5$ in summer and 2.26 x $l0^6$ germs/mL in autumn) and an average of $1.2x10^6$ S. aureus/mL.

In addition, in milk the yeast load varies through the seasons of $4.3x10^4$ cfu/mL in spring to $2.56x10^6$ cfu/mL in summer and $7.58x10^5$ cfu/mL in autumn. This important value of these micro-organisms of deterioration is due to the temperature of summer. Finally we insist with the dangers, which milk resulting from the cows feeding from domestic waste can present.

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