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Physico-Chemical and Bacteriological Qualities of Raw Milk from Collection Centers and Hawking Activity in the Rabat-Salé-Kenitra Region (Morocco)

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Abstract

The study aims to analyze the two circuits of the dairy chain: authorized and unauthorized, by studying the parameters influencing the physico-chemical and bacteriological quality of milk in both networks. Samples of raw milk (n=22), collected from nine collection centers (CCL): four authorized and five unauthorized in the region of Kenitra, and four hawking points in the region of Rabat, during a period between March and May 2022, were analyzed to evaluate their physicochemical and bacteriological qualities. The physicochemical quality of the milk included the measurement of chlorides, lactose and fat content, and the determination of titratable acidity and freezing point. The bacteriological quality was assessed by counting the total aerobic mesophilic flora (TAMF), total and fecal coliforms, presumed pathogenic staphylococci and searching for Salmonella. The fat content varies considerably depending on where the milk samples are collected; the average is 4.13% for licensed collection centers, versus 2.94% for unlicensed collection centers and 2.82% for hawking activity. In terms of hygienic quality, the average bacterial load of TAMF in milk from the authorized collection centers is 3.8×10^{8} CFU/ml. Total coliforms (TC) are present with an average rate of 9.37×10^{5} CFU/ml with an average portion of 7.87×10^{3} CFU/ml in fecal coliforms (FC), and 1.03×10^{6} CFU/ml for presumed pathogenic staphylococci. On the other hand, milk samples from unauthorized collection centers and hawking activity showed higher levels of microbial contamination than those from licensed collection centers. Salmonella was not detected in any of the samples analyzed. The non-compliance with hygiene practices and the late application of cold during the milking, the collection, and the transportation of raw milk, partly explain the origin of this finding.

Keywords: *Milk collection centers, Hawking, Physico-chemical quality, Bacteriological quality*

1. Introduction

In Morocco, several efforts have been made to develop the dairy sector and encourage local production of milk, through the "*Premier Plan Laitier*" project in 1975, the "*Maroc Vert*" plan in

2008 and finally the Green Generation plan in 2020, whose objectives are: The protection of the dairy sector through the taxation of imported powder, the constitution of a core of dairy cattle (Artificial insemination, crossbreeding, ...), the promotion of fodder crops and the establishment of milk collection centers to gather the atomized production, reduce the costs of logistics, and control the quality of milk before its delivery to the industry (Srairi, 2016).

The milk is transported through two main channels: a formal channel of approximately 2,800 collection centers, of which 385 are sanitarily licensed and an informal hawking channel where raw milk is supplied by producers and delivered directly to the various users (households, coffee shops, dairies, etc.) without any heat treatment or quality control (Cherii, 2022).

The control of microbiological and chemical hazards related to milk in the collection centers are controlled within a framework of co-regulation between professionals and the health authority (ONSSA). The professionals are responsible for self-control and the competent sanitary authority is responsible for official monitoring.

2. Materials And Methods

This work includes a physico-chemical analysis of 13 samples from the milk of four authorized collection centers, five unauthorized collection centers and four hawking outlets and a bacteriological analysis of nine samples: three from each network studied (authorized collection centers, unauthorized collection centers and hawker activity). At each collection center and hawking point, a sample of 500 ml was taken after mixing all the milk received and placing it in sterile plastic bottles, which were then kept in a cooler equipped with eutectic plates. The samples were analyzed in the chemistry and microbiology laboratories of the Food Safety Unit (HIDAOA) at the Hassan II Agronomic and Veterinary Institute, Rabat, Morocco.

Physico-chemical analysis:
The physico-chemical executed tests were as follows: determination of titratable acidity (NM ISO, 1995); chloride dosing by the CHARPENTIER-VOHLARD method (Nielsen, 2010); milk fat dosing by the GERBER method (AOAC, 2002); and determination of the freezing point and lactose content by the Lactoscan SA. To ensure accuracy, all physico-chemical tests were repeated three times.

Bacteriological analysis:
The bacteriological quality of the milk was evaluated by enumeration of the Total aerobic mesophilic flora (TAMF) on Plate Count Agar medium after incubation at 30°C for 72 hours (de Normalisation, 2001); enumeration of total coliforms (TC) and fecal coliforms (FC) on Violet Red Bile Agar after incubation at 30°C for TC and at 40°C for FC for 24 to 48 hours (NM ISO, 2004) and the enumeration of presumed pathogenic staphylococci on the Baird Parker Agar with egg yolk and potassium tellurite after incubation at 37°C for 48 hours (NM ISO, 2008), and the search for *salmonella*, carried out using the horizontal method (NM ISO, 2017).

3. Results and Discussion

Physico-chemical quality
The results obtained for the physico-chemical quality are presented in Table 1. The average values recorded for titratable acidity for the three categories exceed the recommended limit of 20 °D, which indicates poor hygiene and significant proliferation of microorganisms (not enough cooling, long transportation time ...). When the acidity of milk reaches 30 to 40 °Dornic (case of licensed collection center A1, unlicensed collection centers NA3, NA4 and NA5), the milk coagulates on heating, and it is therefore, unsuitable to produce pasteurized milk, sterilized UHT, condensed milk and milk powder (Karib, 2023).

The average chloride levels for licensed and unlicensed collection centers are equal, but the average for hawking points is 0.02% higher. These levels are exceeding the threshold of 1.2 g/l because of the addition of salt water to the milk (Godfrain *et al*., 1965) or the presence of mastitic milk in the milk mixture (Benali, 2022).

The fat content is of average quality for the unauthorized collection centers and hawking points except for CP3 whose chloride content is high, which supports the hypothesis of the addition of salt water for this case. For the authorized collection centers, the fat content is excellent for all four centers, and their average is higher than the averages recorded at the collection centers in the Fquih Ben Saleh region (3.71%), the Kssiba region (3.83%) and the Kelaa region (3.34%) (Bassbasi *et al*., 2013).

The averages fat content of authorized and unauthorized collection centers, and hawking activity are higher than Tir's (2015) results, which average only 25.14 g/l. The lactose content of milk from different stages of lactation ranges from 4.52% to 4.53% (SASSI *et al*., 2018). The averages of lactose content registered in this study (between 4.78% and 5.03%) largely exceed the results reported by Bouterfa (2020) and Wendmisida (2013) and which are 4.2% and 4.44% respectively. Regarding the freezing point, and according to Bender (2014), it's between -0.53 and -0.56 °C for milk. The freezing point is the reference test for detecting watered-down milk. Our results sometimes exceed this range (case of: unlicensed collection center NA5, hawking points CP1, CP2 and CP3) because of possible watering-down.

Table 1: Values of the physico-chemical parameters analyzed

	Titratable Acidity (°Dornic)	Chloride (%)	Fat (%)	Lactose (%)	Freezing Point (°C)
A1	30.66	0.13	3.3	4.7	-0.56
A2	28.5	0.17	4.6	4.61	-0.54
A3	22.4	0.12	3.8	4.89	-0.54
A4	26.1	0.11	4.8	4.9	-0.57
Average result of As	**26.91**	**0.13**	**4.13**	**4.78**	**-0.55**
NA1	20.33	0.16	4.2	4.69	-0.55
NA2	20.1	0.13	2.8	4.9	-0.57
NA3	35.66	0.13	2.4	4.87	-0.56
NA4	32.83	0.13	2.8	4.96	-0.55
NA5	33.17	0.12	2.5	5.31	-0.61
Average result of NAs	**28.42**	**0.13**	**2.94**	**4.95**	**-0.56**
PC1	24.5	0.13	3.6	5.12	-0.59
PC2	20.1	0.16	3.2	5.04	-0.58
PC3	19.66	0.17	1.5	5.56	-0.62
PC4	18.5	0.12	3	4.38	-0.50
Average result of PCs	**20.69**	**0.15**	**2.83**	**5.03**	**-0.57**

A: Licensed milk collection center; NA: Unlicensed milk collection center; PC: Hawking point
Source: Calculated by Authors

Microbiological Quality

The results obtained are listed in Table 2. The analysis of these results highlights the presence of significant variations between the different samples of raw milk studied and this for the four groups of bacterial flora counted. The growth rate of the TAMF is closely related to the initial number of germs, temperature, and duration of conservation (FAO, 1995). Therefore, with high initial contamination, and in the absence of early refrigeration of milk, the total flora has enough time to multiply rapidly. There is a positive correlation between the distance between farmers and collection centers and the content of total aerobic mesophilic flora (Gran *et al*., 2002).

This average contamination is in the range of 10^8 and 10^9 CFU/ml in milk from licensed and unlicensed collection centers, respectively. Lower loads were found in the studies of Rahho (Rahho, 2005) and Bouzaid *et al*. (2012), who reported overall contamination in the order of 10^7 CFU/ml. This indicates that the total flora content of Moroccan milk is still higher than the French and American standards (3×10^5 and 5×10^5 CFU/ml respectively), in the absence of a standard in Morocco for raw milk. For authorized LCCs, the average contamination by TC and FC is respectively around 10^5 CFU/ml and 10^3 CFU/ml. For the unauthorized LCCs and the hawking activity, their average contamination by TC and FC is respectively around 10^6 CFU/ml and 10^4 CFU/ml. The presence of coliforms indicates fecal contamination due to poor hygiene or mastitis (Vernozy-Rozand & Roze, 2003).

Assuming that all milk samples were collected under the same milking conditions, the high level of coliforms in the samples can be attributed to the hygiene of the milk storage equipment (buckets, cans and tubs), the duration and the temperature of the milk during transport (Siousarran, 2003). For TC, the results obtained are close to those found by Rahho (Rahho, 2005) which recorded an average of about 10^5 CFU/ml. However, for FC, results lower than ours were found by Rahho (2005) which recorded an average contamination rate of 6.1×10^2 CFU/ml respectively. While, in the study carried out by Bouzaid *et al.* (2012), the contamination rates of FC found are higher than our results, with an average of 1.91×10^5 CFU /ml.

To avoid these contaminations, it is necessary to apply good hygiene practices at the farms, the transportation and at the time of consumption: hygiene of milking, dairy dishes, and staff ..., to keep the milk cold (<6 °C) while avoiding the break of the cold chain, and to prepare dairy derivatives from pasteurized milk. The average levels recorded for presumed pathogenic staphylococci are around 10^6 CFU/ml, which may indicate breast infections (clinical or subclinical).

Contrary to the study done by Bouzaid et *al*. (2012) which reported a total absence of *Staphylococcus aureus* in all peddle milk samples in the city of Rabat, we found a significant contamination, whose average in licensed collection centers is very close to that found by Ait Bacha (2010), and which was 1.3×10^6 CFU/ml. Staphylococcal poisoning is caused by enterotoxins (ETs) that influence the intestinal tract. Typical symptoms are sudden vomiting often preceded by headache, accompanied or not by diarrhea and abdominal cramps that diminish after 12 to 72 hours (Cretenet *et al*., 2011).

To avoid these intoxications, periodic screening for mastitis (clinical examination, CMT test, cell count) should be performed, and then the animals with clinical mastitis should be isolated and treated, and the milk of those with subclinical mastitis should be discarded (Hamama, 2019).

Furthermore, *Salmonella* spp. was absent in all samples analyzed. The isolation of *Salmonella* from milk and dairy products in Morocco is exceptional; indeed, few studies have been able to demonstrate its presence. *Salmonella* spp. was detected in 6.6% of the samples of different naturally fermented milk products made from raw milk in Rabat and in 29% and 11% respectively during the summer and winter periods in the raw milk blend received in two dairy establishments (Hamama, 1990).

The search for *Salmonella* from milk and dairy products has not been performed in many bacteriological studies conducted in Morocco; like the study of the hygienic quality of milk from Sardi breed sheep (Bouazza, 2012), milk from hawking activity in Kenitra (Hadrya *et al*., 2012) and raw milk from dairy farms in Beni Mellal (Hnini *et al*., 2018). Food poisoning related to milk and dairy products is often caused by the consumption of raw milk products or those that have been insufficiently heated (Augustin *et al*., 2020). This is likely due to the presence of *Salmonella* at amounts below the limit of detection (Karraouan *et al*., 2010). The absence of *Salmonella* in fermented dairy products could be attributed to their high acidity level (Al-Rifai *et al*., 2019).

Table 2: Total aerobic mesophilic flora, total and fecal coliforms and presumed pathogenic staphylococci

	TAMF (UFC/ml)	**Staphylococci (UFC/ml)**	**Total Coliforms (UFC/ml)**	**Fecal Coliforms (UFC/ml)**
A2	2.2×10^8	1.24×10^6	1.02×10^6	3.1×10^3
A3	5.8×10^8	1.54×10^6	5×10^5	8.18×10^3
A4	3.4×10^8	3.2×10^5	1.29×10^6	1.23×10^4
Average result (As)	$\mathbf{3.8\times10^8}$	$\mathbf{1.03\times10^6}$	$\mathbf{9.37\times10^5}$	$\mathbf{7.87\times10^3}$
NA3	6.8×10^8	3.7×10^6	1.62×10^6	1.93×10^4
NA4	1.97×10^9	5.3×10^6	1.55×10^6	1.3×10^3
NA5	8.7×10^8	1.39×10^6	1.86×10^7	110×10^3
Average result (NAs)	$\mathbf{1.17\times10^9}$	$\mathbf{3.46\times10^6}$	$\mathbf{7.2\times10^6}$	$\mathbf{4.35\times10^4}$
PC1	7.25×10^8	6.4×10^6	8.2×10^6	3.29×10^4
PC2	5.3×10^8	2.28×10^6	1.63×10^7	1.16×10^4

PC3	1.43×10^9	4.1×10^6	3.8×10^6	2.53×10^4
Average result (PCs)	$\mathbf{8.95\times10^8}$	$\mathbf{4.26\times10^6}$	$\mathbf{9.43\times10^6}$	$\mathbf{2.32\times10^4}$

A: Licensed milk collection center; NA: Unlicensed milk collection center; PC: Hawking Point
Source: Calculated by Authors

4. Conclusion

The physico-chemical quality of the samples is quite good, and the fat content is excellent for the authorized collection centers, but very poor for the milk from the hawking activity whose staff skims the milk to manufacture its derivatives. This study also revealed the current situation of the microbiological quality of the milk mixture. The lowest average contamination rate, for all the microbiological cultures studied, is from authorized collection centers; while the highest average rate is recorded in unauthorized collection centers for TAMF and FC, and in the hawking activity for TC and staphylococci. This excessive charge can be explained by the lack of enforcement of good hygiene practices during the milking, transport, and storage of milk. It is therefore necessary to reinforce the control of authorized collection centers and encourage unauthorized collection centers to respect hygiene practices while following a co-regulation approach.

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Conflict of Interest: The authors of the present study declare that there is no conflict related to this work, which can negatively impact its publication.

Ethical Considerations: All ethical issues (including keeping the identity of the authorized and unauthorized collection centers and hawking points anonymous, double publication and/or submission, and redundancy) have been checked and approved by all authors.

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