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*Full Length Research Paper*

# Microbiological and Physico-chemical Proprieties of Raw Sheep Milk from Sardi Breed

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**Fifty-four samples of raw sheep milk (moroccan Sardi breed) were collected in stables of 18 farms from different regions in Morocco and were analyzed for their microbiological and physico-chemical parameters. The temperature of raw milk just been collected from sheep is  $36.9\pm 0.2^{\circ}\text{C}$ , its pH is  $6.54\pm 0.14$  and titratable acidity is  $23.1^{\circ}\text{D}$ . The average freezing temperature is between  $-0.57^{\circ}\text{C}$  and  $-0.56^{\circ}\text{C}$ . Raw sheep milk had a variable charge of FMAT  $4.5\times 10^3$  to  $9.4\times 10^7$  cfu/ml,  $1.2\times 10^5$  cfu/ml of total coliforms and  $2.10^4$  cfu/ml of fecal coliforms. However, all milks are free of Salmonella, Shigella, *Listeria monocytogenes* and Clostridia. The average fat content is  $67.85\pm 6.55$  g/l. The dry matter content is between 153 and 183 g/l, while the ashes are from 6.9 to 8.7g/l. The lactose concentration varies between 34.5 and 45.34g/l and the calcium content is  $1.84\pm 0.06$ g/l. The results of this study indicate that the microbiological quality of raw sheep milk samples from Sardi breed is below international standards. Low levels of coliforms and *Escherichia coli* as well as the total absence of Salmonella, Shigella and Clostridia indicate that milk is good, and reflect also the good production condition and the respect of good hygienic practices during milking.**

**Keywords:** Sheep, Sardi breed, raw milk, physico-chemical characteristics, hygienic quality.

## INTRODUCTION

Milk is a highly nutritious food for its richness in carbohydrates, fats, vitamins and minerals (Blowey and

Edmondson 2000; Jones 1999). It is a major food taken by populations in arid and semi-arid regions of the world; it is often consumed raw or after processing.

In Morocco, milk and its derivatives are an important source of animal protein for the population. Thus, the individual consumption of milk and dairy products is highly

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different. On average, 81±32kg of milk consumed per person annually is distributed as follows: 36 kg (pasteurized drinking milk or UHT), 17kg (yoghurt), 11kg (unpasteurized milk), 8.9kg (cheese), 8kg (butter) and 0.1kg (leben-curd) (Srairi 2011). According to data from the Ministry of Agriculture and Maritime Fisheries (Sraïri et al., 2009; Sraïri and Karbab 2010), milk production does not cover the needs of citizens, which forces the state to allow the import milk as powder and its derivatives. The search for new sources of milk becomes increasingly essential.

Consumption of sheep milk dates back to thousands of years before Jesus Christ in the Middle East. A fresh sheep milk has all the vitamins and food supplements are highly appreciated (Baltadjieva et al., 1982).

Richer in protein and fat, sheep milk has a soft and slightly sweet taste. It has a dry matter content much higher than cow and goat milk and contains in double fold minerals such as calcium, phosphorus and magnesium and any of the vitamin B group (Baltadjieva et al., 1982). Sheep milk has an advantage for people with allergies or intolerances to cow milk. In addition, (particles of fat) of sheep milk are thinner and therefore more digestible than cow milk. It seems to be so perfect for older people or children that follow special diets (Verdier-Metz et al., 2009).

Of its biochemical composition, milk and its derivatives constitute a favorable environment to the growth of microorganisms specially pathogenic bacteria and fungi; this is why they have always been considered as one of the main causes of food poisoning (Mennane et al., 2007; Grant et al., 2002). Therefore, knowledge of the chemical and microbial composition of the milk is of special interest especially for farmers and processors of milk. The price of the latter is calculated based on the total number of bacteria that should be as low as possible (Verdier-Metz et al., 2009).

Production must therefore be strictly controlled, because of the potential risks it may pose to human health.

Very popular nationally, the Sardi breed occupies a special place in farming in Morocco. It especially dominates regions in the triangle formed by Beni Mellal, Khouribga and Settât (regions of Chaouia Ouardigha and Marrakech Tensift El Haouz). Its membership exceeds 2.1 million herds in 1998, representing 13% of total national herd (Transfert de 2007). Indeed, a study on Moroccan dairy sheep breeds showed that daily milk production of a Sardi sheep is higher compared to other breeds (Boujenane 1995). However, to our knowledge, a comprehensive study on the hygienic quality and chemical composition of sheep milk from Sardi breed has yet been the subject of scientific publication.

The main objective of this research, which is the first approach to the study of sheep milk in Morocco, is to assess the hygienic and physico-chemical quality of raw sheep milk from Sardi breed collected from midsize stables of 5 regions in Morocco, namely: El Gharb Charda Beni

Hssen (EL Mnasra), Rabat-Sale-Zemmour-Zair (Shoul, Zair and EL Gnzra), Casablanca (Oulad Hriz), Chaouia ouardigha (Benhmed, Bni Meskin) and Marrakech-Tensift-El Haouz (Marrakech, El Kelâa of Sraghna).

## MATERIALS AND METHODS

### Sampling

Analyzed samples are whole raw milks of small mix from 5 Sardi ewes, for each sample, in early lactation (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> months of lactation). Ewes are aged from 1 to 3 years (a total of about 300 females were treated in this study, belonging to herds in 18 rural and suburban farms regions).

Ewes were randomly taken for milking. It was done manually in March 2014, in the morning, before the herds were released for grazing. The milk was collected in sterile bottles and transported in a cooler under a temperature of 4°C in the dark to the laboratory. All analyses were performed in duplicate for each sample; the values shown in the tables are averages of the results obtained for samples already mentioned for each region.

### Microbiological Analysis

For each sample, 10ml of raw sheep milk was added into an Erlenmeyer flask to 90ml of sterile saline. Thus, a mother dilution of 10<sup>-1</sup> was obtained, from which decimal dilutions of 10<sup>-7</sup> were performed.

Total aerobic mesophilic flora (FMAT), a good indicator of contamination, is counted on PCA agar incubated for 48 h at 30°C.

Coliforms are sought on desoxycolate lactose agar (DL) incubated for 24 h at 37°C for total coliforms and 44°C for fecal coliforms. Fecal streptococci were enumerated on sodium azide after 48 h of incubation at 37°C. Staphylococci are enumerated on Baird Parker agar added to the egg yolk and of potassium tellurite; and then incubated for 48 h at 37°C.

For *Salmonella*, a pre-enrichment using selenite cysteine medium for 12 h at 37°C was done, followed by an enrichment using broth Tetrathionate for 24 h at 37°C. Then enumeration and isolation were performed on the medium SS (*Salmonella-Shigella*) after 24 h of incubation at 37°C.

Sulphite-reducing clostridia are counted in the reinforced *Clostridium* Agar medium culture in tubes to promote anaerobic conditions, with heat treatment for 10 min at 80°C and also to activate the clostridia spores. They can persist in a latent form in milk, germinate when conditions are favorable and secrete toxic substances. The tubes are

incubated for 48 h at 37°C. Only black colonies are counted.

Yeasts and molds were counted on Sabouraud dextrose medium at 4% and incubated for 5 days at 25°C. Lactic acid bacteria are enumerated on MRS agar medium (Man Rogosa Sharpe, Difco, Detroit, USA), M17 (Oxoid) and Elikor (Oxoid) and incubated for 48h at 30°C.

### **Physico-chemical Analysis**

#### **Physical Parameters**

##### **Temperature**

Just after milking, the milk temperature is measured with a thermometer.

##### **PH**

One of the raw milk samples was taken to the laboratory. The pH was measured at 20°C using a pH meter Orion Type after calibration with Ph 7.00 and 4.00 by soaking in a small volume milk collected in a beaker.

##### **Titrateable Acidity**

This parameter is measured by titration with NaOH Dornic Soda N/9N in the presence of phenolphthalein and is expressed as percentage of lactic acid. A specific sample of 10ml of milk was placed in a 100 ml beaker with 0.1ml of 1% phenolphthalein in 95% alcohol. Dornic Soda (N/9) is added (in the burette) for it to turn pink. The pink color should persist for at least 10 seconds (GUIRAUD 1998).

##### **Density**

The density is measured using a thermo couple on the milk lacto densimeter kept standing. The idea is to immerse a hydrometer in a test tube filled with 100 ml milk sample. When stabilized, direct reading, gives us the result. The density is measured at 20°C.

##### **Freezing Temperature**

The freezer temperature was determined using an incubator/ freezer (BIO Firlabo).

##### **Chemical Analysis**

##### **Dry Matter and Ash**

We introduce in a pre-weighed capsule 10ml of milk using a volume tric pipette. Then it was placed in a set at 103±2°C for 3 h. After drying, the capsules were weighed

and the difference between the two weights was multiplied by 100. Ash content, expressed in gram per liter of milk, was determined after drying at 550°C.

##### **Fat**

The fat content is determined by the acid-butyrometric Gerber's method (AFNOR standard NF V04-210). It consists of milk attacked by sulfuric acid and separated by centrifugation in the presence of alcohol isoamyl fat released. Milk proteins are dissolved by the sulfuric acid, are resistant to the action of concentrated sulfuric acid fat and are separated when hot by centrifugation, in the presence of isoamyl alcohol (3-methyl-1-butanol), which facilitates separation. We measure the volume from 65 to 70°C in a Butyrometer of Gerber (Granville and Desmet 1951).

##### **Dosage Lactose**

Lactose is expressed in gram per liter and is determined by the Bertrand method (Granville and Desmet 1951).

##### **Determination of Calcium**

The dosage of Calcium (Ca) is performed using atomic absorption spectrometer in the presence of lanthanum chloride (Sigma Chemical, St Louis USA) (Afnor, 1993).

## **RESULTS**

### **Microbiological Analysis**

The raw sheep milk analyzed contains a variable charge of FMAT, between  $4.5 \times 10^3$  to  $9.4 \times 10^7$  cfu/ml (Table I), reflecting the variability of milking practices from one farm to another. The highest rate was observed in the samples that originated from El Kelâa of Sraghna. Indicators of general hygiene and fecal contamination, total and fecal coliforms are present at low or missing values in most samples. Their number does not exceed  $1.2 \times 10^5$  (CFU/ml) for total coliforms and  $1.5 \times 10^4$  for fecal coliforms.

The analysis revealed samples contaminated with streptococci. The average content varies from  $1.1 \times 10^2$  CFU/ml with an average value of  $0.4 \times 10^3$  CFU/ml. *Staphylococci*, *Salmonella*, *Shigella* and *Clostridia* were totally absent in all samples.

The average charge of lactic bacteria is  $8.1 \pm 1.33 \times 10^5$  CFU/ml, with fluctuations ranging from  $5.0 \times 10^5$  to  $9.5 \times 10^5$  CFU/ml. The average yeast load is  $1.2 \times 10^4$  CFU/ml.

Table I. Microbiological analysis (cfu / ml) of raw sheep milk of samples tested

SAMPLES		FMAT	COLIFORMS		STREPTOCOCCI	YEASTS	LACTIC BACTERIA
			Total coliforms	Fecal coliforms			
Region of El Gharb	EL MNASRA	$4,5 \cdot 10^3$	<10	<10	$0,5 \cdot 10^2$	$2,6 \cdot 10^3$	$7,9 \cdot 10^5$
Region of Rabat-Sale-Zemmour Zair	Shoul	$1,2 \cdot 10^4$	<10	<10	<10	$0,2 \cdot 10^4$	$7,7 \cdot 10^5$
	Zair	$2,3 \cdot 10^4$	<10	<10	$0,1 \cdot 10^2$	$2,3 \cdot 10^4$	$8,3 \cdot 10^5$
	El Gnzra	$7,4 \cdot 10^4$	<10	<10	$0,3 \cdot 10^2$	$1,3 \cdot 10^3$	$9,5 \cdot 10^5$
Region of grand Casablanca	Oulad Hriz	$5,3 \cdot 10^6$	<10	<10	$1,1 \cdot 10^2$	$1,1 \cdot 10^4$	$9,2 \cdot 10^5$
Region of Chaouia Ouardigha	Ben Hmed	$8,2 \cdot 10^4$	<10	<10	$0,2 \cdot 10^2$	$0,5 \cdot 10^5$	$8,3 \cdot 10^5$
	Beni Meskin	$6,1 \cdot 10^5$	<10	<10	$0,1 \cdot 10^2$	$2,8 \cdot 10^3$	$8,7 \cdot 10^5$
Region of Marrakech Tensift El Haouz	Marrakech	$6,4 \cdot 10^7$	$1,2 \cdot 10^5$	$1,5 \cdot 10^4$	$3,6 \cdot 10^2$	$3,7 \cdot 10^4$	$9,0 \cdot 10^5$
	El Kelâa of Sraghna	$9,4 \cdot 10^7$	$1,2 \cdot 10^5$	$2 \cdot 10^4$	$4,1 \cdot 10^2$	$7,4 \cdot 10^4$	$5,0 \cdot 10^5$

### Physico-chemical Analysis

The results of the physico-chemical analysis are shown in Table II.

Temperatures measured immediately after milking are between 36.7 and 37.1°C (Table II). The pH varies from 6.43 to 6.65, with an average of 6.54. The value of titratable acidity is 23.1°D. Density measured at 20°C is between 1.032 and 1.037 g/L, with a mean value of 1.034 g/L.

The average fat content is 67.45 g/L. The average freezing temperature is -0.57°C. The solid content is between 153 and 183 g/L, with an average of 168 g/L. The average concentration of lactose is 40.9 g/L, where as the calcium content is  $1.84 \pm 0.06$  g/L.

### DISCUSSION

The total aerobic mesophilic flora (FMAT), payment base for milk quality, is considered as a general indicator of the overall product quality. It reveals the conditions of production, particularly hygienic practices during milking.

The results obtained through this study indicate that samples of raw sheep milk analyzed are loaded with more

microorganisms than cow and goat milk:  $3.5 \times 10^2$  CFU/ml on the day of packing and  $3 \times 10^4$  CFU/ml on the deadline for consumption, according to (Joffin and Joffin 1999). According to (Dacosta 2000) and (El-Shafie et al., 2008), sheep milk has high antibacterial properties that ensure cool conservation without immediate fermentation. This finding opposes the relatively high microbial load in the samples analyzed. In this sense, (Calvo and Olano 1992) reported that when milk is collected under appropriate hygienic conditions, the total flora does not exceed  $10^3$  to  $10^4$  CFU/ml. Indeed, 70% of sheep milk from Sardi breed collected did not exceed the European standards ( $500 \times 10^3$  CFU/ml for raw milk intended for the manufacture of raw milk products,  $1500 \times 10^3$  CFU/ml for milk intended for heat treatment) laid down by Directive 92/46 (Anonymous 1992). These results are similar to those found by several authors in various countries: Switzerland (Muehlerr et al., 2003; Zweifel et al., 2005). Spain (Gonzalo et al., 2006). North-eastern Greece (Alexopoulos et al., 2011), and Morocco (Bouazza et al., 2012). Noting that, in our case, the highest load was detected in samples of El Kelâa of Sraghna. The importance of this high microbial contamination observed in sheep milk collected from Marrakech-Tensift-El Haouz is due to several factors:

Table II. Physico-chemical analysis of raw sheep milk of samples tested

Physical Parametrs		Milk after milking (°C)	pH	Acidity (%)	Density of whole milk at 20°C	fat (g/l)	Dry extract (g /l)	Freezing temperature (°C)	Lactose (g/l)	Calcium (g/l)
Region of Gharb	EL MNASRA	36,9	6,45	24,5	1,036	68,7	168	-0,56	42,43	1,87
Region of Rabat-Sale-Zemmour Zair	Shoul	36,9	<b>6,65</b>	22, 2	1,034	67,5	164	-0,57	40,9	1,86
	Zair	36,8	6,61	<b>21,1</b>	1,035	67,6	160	-0,57	38,5	1,80
	El Gnzra	36,9	6,59	22,1	1,034	66,6	154	<b>-0,57</b>	<b>34,5</b>	1,84
Region of Casablanca	Oulad Hriz	<b>36,7</b>	6,55	22,5	1,035	<b>61,3</b>	<b>153</b>	<b>-0,56</b>	37,45	1,88
Region of Chaouia Ouardigha	Ben Hmed	36,8	6,49	23,9	1,036	73,7	183	-0,57	43,27	1,89
	Bni Meskin	36,9	6,55	24,6	<b>1,037</b>	<b>74,4</b>	<b>185</b>	-0,57	<b>45,34</b>	<b>1,9</b>
Region of Marrakech Tensift Haouz	Marrakech	36,9	<b>6,43</b>	<b>25</b>	1,033	65,8	181	-0,57	42,26	1,81
	El Kelâa of Sraghna	<b>37,1</b>	6,57	24,9	<b>1,032</b>	64,9	173	-0,57	39,40	<b>1,78</b>

hygiene during milking, transport conditions and favorable growth temperatures for microorganisms during their transport to the testing laboratory.

The search for indicators of fecal contamination from micro-organisms can be used to judge the hygienic condition of the product. Even at low levels, they also testify to the deterioration of hygienic conditions during milking or during transport. Levels of coliforms found are comparable to those reported by (Benalia et al., 2013):  $1.1 \times 10^5$  for total coliforms CFU/ml and  $1.5 \times 10^4$  for fecal coliforms. Milk has low and normal microbial over all burdens, while milking technique is traditional. Coliforms seen only in the sheep milk collected from Marrakech-Tensift-El Haouz is due to trafficking and the distance to get to the laboratory. Despite this, the contents of coliforms (total, fecal) found are lower than those provided by (Gasmi-Boubaker et al., 2013).

The rate of streptococci is related to the health of the sheep, the hygienic conditions of trafficking, and possible contamination during enumeration. In fact, in our case, this rate does not exceed the values recommended by the Moroccan and international standards. The yeast load between  $1.3 \times 10^3$  and  $0.5 \times 10^5$  CFU/ml is normal and it will allow necessary fermentation to produce milk derivatives.

The raw sheep milks tested have a very good microbiological quality and hygienically, they are acceptable. The absence of salmonella, staphylococci and clostridia indicates a healthy sheep selected from stables and also a healthy trafficking (Hamama and Bayi 1991; Pollma and Sillerker 1984).

PH tells of the milk freshness precisely. The average pH values of raw sheep milk from Sardi breed analyzed range between 6.43 and 6.6. These values found in this study are similar to those reported by some authors such as (Delacroix-Buchet and Marie 1994) and (Baltadjieva et al., 1982) on sheep milk from French Lacaune breed and Plovdiv regions in Bulgaria and Ioannina in Greece. Indeed, compared to human and cow milk, sheep milk is slightly acidic. In this context, Labioui (2009) evaluated the pH of raw cow milk in the region of El Gharb and gave average values of  $6.70 \pm 0.15$ . These values are lower than those found by (Mathieu 1998), for ten samples of cow milk collected under traditional milking conditions. The highest value is marked in samples that originated from Shoul (6.65) in the Region of Rabat Sale while the lowest value is recorded in the samples collected in Marrakech (6.43). The pH also depends on the presence of casein, phosphoric anions and citric acid.

Titration acidity values are high compared to those of cow milk (Bennacir 1980). The average of 23.1°D remains never the less in the range of 21 to 24°D of fresh milk. This parameter is low in samples from Rabat-Sale and higher in those from Marrakech stables. Acidity depends mainly on the casein content, minerals and ions (Alais 1984); it also depends on hygienic conditions during milking, total microbial flora and its metabolic activity (Mathieu 1998) and on handling of milk.

The average density values (1.032 to 1.037 g/L) are similar to those reported by (Baltadjieva et al., 1982). (1.033 to 1.038 g/L). For cons, the sheep milk density

(1.028 to 1.034 g/L) appears to be higher than that of raw cow milk (Mathieu 1998). The average of the highest density is observed in Chaouia Ouardigha Region. However, this parameter depends on the dry matter content, fat content, the increase in temperature and food availability. (Baltadjieva et al., 1982), also reported that during lactation, density and acidity of milk fluctuate slightly by region (Baltadjieva et al., 1982).

Sheep milk is richer than cow and goat milk. In our case, the average dry matter content is in the order of 168 g/L against only 121.7g/L for cow milk (Labioui et al., 2005), while the ashes about 6.9 and 8.7g/L. The value of the dry extract is in the range of international standards, depending on climatic and dietary factors.

On average, sheep milk contains 61 to 75g/L of fat. This content is confirmed in this study and seems higher compared to cow milk (31.45 g/L), according to Labioui (Labioui et al., 2005). The higher fat content of sheep milk is noted in the samples collected in Ben Hmed and Bni Meskin from Chaouia Ouardigha Region, while the lowest fat content is marked in samples collected in Oulad Hriz from Casablanca Region. In fact, the variability of the fat content also depends on factors such as weather conditions, lactation stage and power supply (Seboussi et al., 2010).

From the results compiled in Table II, the average lactose content of the raw sheep milk from Sardi breed varies between 34.5 and 45.34g/L. These levels appear lower than those of cow milk(49.00 g/L) (Mathieu 1998; Labioui et al., 2005). Lactose, the main sugar found in milk and substrate of lactic fermentation for lactic acid bacteria, is in the normal range for raw milk(40 to 50g/L).The high lactose content of the sheep milk is observed in Chaouia Ouardigha Region (Beni Meskin), while the lowest value is recorded in Rabat-Sale Zair Region (El Gnzra). The lactose content seems to depend mainly on the breed type, the stage of lactation and hydration status (Yagil and Etzion 1980).

The calcium content in sheep milk (1.78to 1.9 g/L) is similar to that reported by (Baltadjieva et al., 1982), (1.86 to 1.94 g/L). Generally, it is also greater than that of cow milk (0.8 to 1g/L). The higher mineral content is observed in samples from Chaouia Ouardigha (1.9g/L), while the lowest value is recorded for samples collected from El Kelâa of Sraghna (1.78g/L).

## CONCLUSION

Traditional milking done in 18 farms allowed us to obtain sheep milk from Sardi breed of good bacteriological quality and which is also high in fat, carbohydrates and calcium. It is used for the production of organic raw milk products of good taste: lbens, yoghurt, fresh cheese, etc.

Variations between regions are important, as they denote a lack of compliance with good manufacturing practices at the trafficking and transport of raw milk. Pasteurization is important and requires the combination of temperature and time. Disinfecting equipment and hygienic milking immediately followed by refrigeration of milk reduce contamination. Timeliness and more rigorous control of the market for veterinary products would reduce the rate of residues. The presence of pathogenic bacteria and residues should be examined with a view to analyzing the risk to consumers. The concerted action of the various actors in the sector, combined with their incentives could improve the quality needs.

Ultimately, this study also shows the effect of variations between morrocan regions, and the difference between the performances of grazing dairy sheep cannot be attributed to the nature of the operation in the strict sense, but depends essentially on modifying the nutrient in take observed during this period. The causes of these changes (especially the intake and/or the nutritional value of grazed grass) as well as director indirect effects of non-food factors (climatic or social) from one region on the performance of the animals need to be confirmed.

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