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ORIGINAL RESEARCH ARTICLE

Biochemical, Physico-Chemical and Microbiological Properties of Camel Raw Milk Marketed in Bechar city (South-West Algeria): Hygienic and Safe Consumers Approach

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ABSTRACT

This study focused on the analysis of twenty-four samples of camel raw milk commercialized at the market of Bechar city. Sample analysis of raw milk was performed by measuring of some physicochemical parameters which the average results obtained have a slightly acidic pH (5.67), high titratable acidity (4.49g/l). Density, total dry extract and fat content were low (1.0268; 89g/l and 29.87g/l respectively), a lactose content of (28.18 g/l). The microbiological examination has several microbial quality attributes such as total bacteria, thermo tolerant coliforms, fecal Streptococci, Sulphite-reducing clostridia and fungal flora with average obtained 3.1 x 10^7 cfu/ml, 4.9 x 10^4 cfu/ml, 1.16 x 10 germ/ml, less than 5 spore/20ml and 2.36 x 10^3 uf/ml respectively. In addition, the analysis revealed the presence of coagulase positive Staphylococci for two samples, however, all samples are free of *Salmonella* sp. and *Shigella* sp. 58% of the samples were of satisfactory quality, 8.33% are acceptable and 33.33% are unacceptable.

.**Key Words:** Camel raw milk, Quality, Health, Consumers, Bechar.

Introduction

During the last decade, the camel has been the subject of special attention from the national and local authorities, a view to its better resistance ability to drought conditions in arid and semi-arid regions and its development (Ben Aissa, 1989; Ellouze and Kamoun, 1989). Currently, there are about 19 million head in the world, with 245,000 heads are present in Algeria (FAO, 2004).

It is used for the supply of milk, meat, skin and transportation (Eberlein, 2007). Camel milk is traditionally valued for its anti-infective, anti-cancer, anti-diabetic properties and more generally as tonics in convalescent patients (Konuspayeva *et al.*, 2004).

This milk presents a physico-chemical composition relatively similar to that of bovine milk. However, it is distinguished by a high content of vitamin C, niacin, and the presence of a powerful protective system, with relatively high levels of lysozyme, lactoperoxidase, lactoferrin and bacteriocins produced by lactic acid bacteria (Siboukeur 2007).

Even from very ancient times, it is the main food source for nomads who usually consuming in its raw state (Kamoun and Ramet, 1989), and despite its natural qualities, camel milk does not escape from contamination problems (Tourette *et al.*, 2002).

Milk is an excellent culture medium for certain bacteria, particularly pathogenic bacteria that can cause organoleptic changes and alter the quality of this product, which requires a review of its hygienic quality.

For this purpose, the present study was undertaken, to review an essential resource that characterizes the region of Bechar namely camel raw milk of the species "*Camelus dromedaries*", and to evaluate its nutritional and hygienic quality by analysis of some biochemical, physico-chemical and microbiological parameters.

Materials and Methods

Sampling

The samples were performed at the market town of Bechar between the period of February to April 2014, with a total of 24 samples. These are placed in a cool box and transported directly to the laboratory for analysis, where they realized in pedagogical biology laboratory of Bechar university (Algeria), whose purpose to assess the biochemical, physico-chemical and microbiological properties of camel raw milk commercialized at the market town of Bechar.

Physico-chemical analysis:

The studied physic-chemical parameters were: temperature, pH, titratable acidity (%), density, total dry extract (%), fat content (%) and lactose content.

The pH and temperature were determined byStarter 2C Lab pH meter, Shanghai Chinaand a mercury thermometer, respectively. The titratable acidity was determined by measurement of lactic acid with sodium hydroxide in the presence of phenolphthalein at 1% as a color indicator according to (NF V04-206, 1994; ISO 11869, 1997). The density of milk was measured by using a calibrated thermo-lactodensometer (AFNOR, 1993) to give (by simply reading the line corresponding to the point of touching) the density of the sample to be analyzed in which he floats. The fat content was measured by the butyrometric of Gerber method according to (NF V 04-210, 1990) which consists of a milk attack by sulfuric acid and separation by centrifugation in the presence of isoamyl alcohol.

Total solid content and water content were determined after drying in an oven at 103± 2°C (drying method) according to (NF V04-207, 1994; ISO 13580, 2005).

The lactose content is determined by UV-visible spectrophotometry (UV-1700). 1 ml of milk, we added 1 ml of phenol-water and 5 ml of sulfuric acid. The assembly is mechanically homogenized on vortex and then brought to a boil for five minutes. The absorbance is read at 490 nm against control sample prepared with distilled water. A calibration curve is constructed from a stock solution of 0.1% of lactose (AFNOR 1993).

Microbiological Analysis:

Sampling was performed according to the Algerian standard (NA 676, 1994). Microbiological analysis included after preparing decimal dilutions according to (ISO 8261, 2001), the detection and enumeration of bacteria indicators of fecal contamination or sanitary quality defects, and the search for suspected pathogenic bacteria, including;

Enumeration of total aerobic mesophyll flora incubated at 30°C for 72h according to (NF V 04-016, 1985) on Plate Count Agar (PCA) (Fluka, Spain); Detection and enumeration of coliform organisms and thermo-tolerant coliform incubated at 37 and 44°C respectively for 24 to 48h according to (ISO 4832, 1978) on the middle Violet Red Bile Lactose Agar (VRBL) (Biochem, Canada). All red colonies (lactose +) with a minimum diameter of 0.5 mm appeared in 24h are regarded as coliforms; Detection and enumeration of *Staphylococcus aureus* according to (ISO 5944, 2001) on the middle of Giolitti Cantoni (Institut Pasteur, Algeria) and Baird Parker Agar (Fluka, Switzerland); Detection of *Salmonella* spp according to (ISO 6785, 2001) after pre-enrichment in non-selective liquid medium and enrichment in selective media (Rappaport-Vassiliadis and selenite/cystine middle) in a deep tube; Search and enumeration in a liquid medium Rothe (Scharlau, Spain) for faecal Streptococci incubated at 37°C for 48h following the method described by (Afif *et al*., 2008). The contents of positive tubes with turbid appearance, were then subcultured on Litsky medium (Institut Pasteur, Algeria) with a platinum loop and subjected to incubation at 37°C for 48h; and finally, enumeration of fungal flora on agar Sabauraud 4% glucose (Fluka, India) according to (ISO 6611, 1996).

Enumeration of Petri dishes having microorganisms is based on the standard set by legislation (AFNOR, 1980).

Identification of bacterial isolates:

The characterization of suspected pathogenic bacteria isolates was performed by the following steps:

- The first step is to examine the morphology of isolates namely *Staphylococcus aureus* and Salmonella sp by macroscopic examination of colonies on nutrient agar and microscopic by microscopic observation in the fresh state and Gram staining.
- The second step was based on the identification of the biochemical characteristics using classical and miniaturized API20 E Gallery kit.

Results

Physico-chemical analysis:

The results for the physico-chemical characteristics of camel raw milk are given in table below (Table 1).

The results of pH show that camel raw milk samples analyzed are slightly acid with an average value of 5.67.

The analyzed samples have an average value of lactic acid 4.49g/l, with variations ranging from 2.06 to 8.7 g/l.

These results appear to be higher compared to those given by the Algerian regulations which vary between 1.4 and 1.8 g/l for fresh milk.

After measuring the density of camel raw milk samples collected, we obtained an average value of 1.0268. These results have identified a few divergence between the samples analyzed.

The total solids content of the samples is equal to 89.06g/l, varying from 62.33 to 121g/l. These results appear to be low, and is accorded positively to density values found which is also low.

The fat content of the samples of milk varies between 16 and 55 g/l with an average of 29.87 g/l.

According to the results compiled in Table 1, the average lactose content of camel raw milk was 28.18g/l, with variations ranging from 12 to 41g/l.

Table 1: Physico-chemical analysis of camel raw milk

Product analyzed	**Parameters analyzed: Average values**						
	pH	Lactic Ac. (g/l)	Density	MG (g/l)	EST (g/l)	ESD (g/l)	T. Lactose (g/l)
Camelraw milk	5.67	4.49	1.0268	29.87	89.065	55.92	28.18

pH (potential of hydrogen); EST (Total Dry extract); Lactic Ac. (lactic acid); ESD (Defatted dry extract); MG (Fat content); T. Lactose (lactose content).

Microbiological analysis:

The results of microbiological parameters are given in Table below (Table 2).

The results of the detection and enumeration of FAMT revealed a high rate whose average value obtained was estimated of $3.1x10^7$cfu/ml.

These values appear to be higher compared to that reported in the Algerian regulation (NA 35, 1998), which recommended a load that does not exceed $3x10^5$cfu/ml for milk has a satisfactory quality.

The results of faecal contamination namely total coliforms showed values ranged from $1.7x10^2$ and $2.7x10^7$cfu/ml, with a total absence of these organisms eventually in three samples.

However, the rate of thermo-tolerant coliform was higher than threshold recommended by the Algerian regulatory which nine samples were contaminated with a load ranging from $5x10^3$ to $1.5x10^7$ cfu/ml.

The rate of fecal streptococci was variable between milk samples analyzed which they had an average of 1.16 x 10 germ/ml, knowing that most of the samples are free of Streptococci except six samples.

The number of clostridium spores was variable between the milk samples. These samples showed an average less than 5 spores/20ml. However, no clostridium spores were detected for twelve samples knowing that all samples are satisfactory for this parameter following Algerian regulations (Under 50 spore/20ml).

Among the analyzed samples of camel raw milk, two samples were contaminated by presumed pathogenic staphylococci *"Staphylococcus aureus"* which seem unsatisfactory according to the rules established, while the other samples were free of this organism with the presence of coagulase-negative staphylococci (SCN) identified as a species of "*Staphylococcus saprophyticus*".

The average values obtained of SCN and presumed pathogenic Staphylococcus were $1.68x10^4$ and $3.75x10^2$ cfu/ml, respectively, knowing that five samples were free of Staphylococcus.

Search for *Salmonella* and *Shigella* revealed a complete absence of these pathogens in the milk sample analyzed. These results comply with the regulatory Algerian established. The fungal flora is present in the analyzed samples with an average of 2.36×10^3 fu/ml.

Table 2: Microbiological analysis of camel raw milk

Product analyzed	Parameters analyzed: Average values							
	FAMT (cfu/ml)	Coliforms (cfu/ml)		Staphylococci (cfu/ml)		Str. fécaux (germes/ml)	YM (uf/ml)	CSR (spore/20ml)
		CT	CF	SCN	*S. aureus*			
Camelraw milk	$3.1x10^7$	$2.8x10^6$	$4.9x10^4$	$1.68x10^4$	$3.75x10^2$	1.16x10	$2.36x10^3$	4.75

FAMT (Total aerobic mesophilic flora); CT (total coliforms); CF (fecal coliform); LM (yeasts and molds); Str. stool (fecal streptococci); CSR (sulphite-reducing clostridia); *S. aureus* (*Staphylococcus aureus*), SCN (coagulase negative Staphylococci); cfu (colony forming unit); uf (fungal unit).

Discussion

Physico-chemical analysis:

The pH is a parameter determining suitability for food preservation. It is one of the main obstacles that the microbial flora must cross to ensure its proliferation. However, a pH of 4.34 to 6.40 is very favorable to the development of fungal flora 'yeasts and molds' and the pathogenic species "*Staphylococcus aureus*". Most of *S. aureus* strains grow at pH between 4 and 10 with a pH optimum between 6 and 7 (Hennekinne, 2009). The average values pH of studied milk are less than those found by Siboukeur (2007) (6.31) and Sboui *et al.,* (2009) (6.41), Abu-Tarboush *et al.,* (1998) in Saudi Arabia (6.49). Other authors have obtained higher values namely Mehaia (2006) in Saudi Arabia (6.62), Kamoun (1995) in Tunisia (6.51), Alloui-lombarkia *et al.,* (2007) in Algeria (6.51). The variability of the results for the samples are mainly related to climate, stage of lactation, food availability, fluid intake and storage conditions of milk (Labioui *et al.,* 2009).

The pH as well as the taste of milk may depend on the nature of the food and water availability (Gorban and Izzeldin, 1997). Saley (1993) estimates that the relatively high vitamin C content of camel milk would be originally of the low pH. In addition, the low pH of the camel milk can be attributed to the high concentration of volatile fatty acid (Yagil, 1985). The pH also depends on the presence of casein and anions of phosphoric and citric acids. According to Labioui *et al.,* (2009), titratable acidity is the sum of four reactions. The first three represent natural milk acidity (acidity due to casein, mineral salts and phosphates), and the latter is related to the acidity "developed", due to the lactic acid and other acids from the microbial degradation of lactose and possibly being altered lipids.

The results obtained here was high acidity. However, many authors report values less than 4.5 g/l, such as Alloui-lombarkia *et al.,* (2007) in Algeria (1.512), Kamoun (1995) in Tunisia (1.56g/l), Sboui *et al.,* (2009) (1.72g/l), Siboukeur (2007) (1.82g/l) and Chethouna (2011) (1.8g/l). The results obtained are comparable to the density values reported by FAO (1995) with 1.026 and appear to be slightly higher compared to data of Siboukeur (2007) (1.0230) and Chethouna (2011) (1.0220). The density depends directly on the dry matter content, strongly related to the watering frequency (Siboukeur, 2007). The density also varies in proportion to the concentration of dissolved components and suspended but inversely to the fat content. It varies with the temperature (Boubezari, 2010).

The solids content of milk also varied depending on the stage of lactation (Bengoumi *et al.,* 1994). As well, it decreases during the month after calving (FAO, 1995). The values obtained are higher than those reported by Bengoumi *et al.,* (1994): (69.5g/l) and less than that reported by Siboukeur (2007) (113.11g/l) and that reported by Chethouna (2011) (102.42g/l). Indeed, the dilution of milk during hot season reflects a phenomenon of adaptation of the camel in the desert and through which the camel is supplemented with sufficient nutrients and water (Musaad *et al.,* 2013). On the other hand, the water content of the milk is affected camel by the water content of the plants ingested camel or fraud by the addition of water by individual traders. The fat content obtained is situated in the range of works cited by Haddadin *et al.,* (2007) with 25 to 35 g/l in Jordan, approximates to those reported by Meiloud *et al.,* (2011) with 29 g/l in Mauritania and lower than those reported by Gorban and Izzeldin (2001) with 32 to 35 g/l and that of Kamal *et al.,* (2007) with 37.8 g/l in Egypt and Tunisia respectively.

The variability of fat content depends on factors such as weather conditions, stage of lactation and food (Labioui *et al.,* 2009). In addition the above mentioned variations are related to the hydration state of the animal and to the power supply. Khaskheli *et al.,* (2005); Elamine and Wilcox (1990) found that other factors such as season, stage of lactation and number of calving are likely to interfere with the values obtained. The rate of lactose registered in this study is lower than those reported by Alloui-lombarkia *et al.,* (2007) (34.20 g/l) and those reported by Mahaia *et al.,* (1995) (43.3 g/l). Other authors have found higher levels namely Siboukeur (2005) (43.87g/l) and Kamal *et al.,* (2007) (58.5g/l). The changes in the levels of lactose are responsible for the sweet taste and at times bitter of camel milk (Yagil, 1982). These variations are very low, depending on the season (Hadaddin *et al.,* 2007). They depend on the race, stage of lactation and also the state of hydration (Ellouze and Kamoun, 1989; Kamal *et al.,* 2007; Siboukeur, 2008).

***Microbiological analysis*:**

To ensure consumer safety, we need controlled food products that we produce, and one wonders, is that we have respected the microbiological criteria of the product during manufacturing? This is because the evolution of the initial microbial flora present in a food depends on many factors related to the physicochemical and biochemical characteristics of the food and the treatment to which the food is subjected. The rate obtained by total aerobic mesophilic flora $3.1x10^7$cfu/ml is higher than that allowed by Algerian standard (NA 35, 1998). This is explained by the results of the pH and Dornic acidity which were correlated with the rate of total isolated germs.

The high microbial load in camel milk is probably due to several factors; poor hygienic conditions during milking or conservation which cause proliferation of milk contaminants and high temperatures in the arid and semi-arid promoting the growth of these microorganisms (Chethouna, 2011). Coliforms are the usual hosts of the mammalian intestine; their presence in milk is an indication of a direct or indirect fecal contamination due to poor hygiene practices during milking or conservation. It is therefore more general markers of hygienic quality. Many coliforms are not dangerous except in case of extremely abundant proliferation (Guiraud and Rosec, 2004). Based on the results of total coliform, it appears that the average obtained is less than those quoted by other work namely Tourette (2002) ($3.55x10^4$ cfu/ml); Benkerroum *et al.,* (2003) (1.6x104 cfu/ml) and is closer to those given by Siboukeur (2007) (10^5 to 10^6 cfu/ml). However, these organisms are absent in three samples. These results may suggest that this flora is absent or is likely inhibited by other factors present in milk such as proteins and peptides with antimicrobial activities that are produced by the lactic flora. Indeed, the presence of these factors in the camel milk and their role has been reported by various authors (Kamoun, 1995).

The limits of acceptability for the presence of thermo-tolerant coliform bacteria in raw milk is $3x10^3$ cfu/ml. Consequently, and in accordance with the standard set by the text cited above, 33% of camel milk samples analyzed are considered as unacceptable quality products where the average of these germs is greater than 10^4 cfu/ml. The data

relating to enumeration of faecal streptococci for camel raw milk analyzed had an average higher than that reported by the Algerian standard (NA 35, 1998) and lower than those found by some authors such as Labioui *et al.,* (2009) ($0.4x10^3$ germ/ml). In addition, the total absence of streptococci in almost all samples except 25% of the samples which have been contaminated. Thermo tolerant coliforms/fecal streptococci report is generally greater than 1, this indicates that there was a faecal contamination (Cuq, 2007). The presence of pathogenic germs namely coagulase-positive Staphylococci *'Staphylococcus aureus*' in both samples suggest the unsatisfactory quality of these samples. By against the presence of coagulase-negative staphylococci in the other samples indicates that there's a lack of hygiene during milking, which is reported by Broutin *et al.,* (2005) that Staphylococci are considered as a witness hygiene.

Thus, the presence of staphylococci in samples of milk collected aseptically reveals that there is an exogenous contamination. It may include Staphylococci present on the udder and joining the milk during milking (wounds, unwashed udder before milking) or Staphylococcus carried by the milker. The absence of salmonella explained by the absence of the origin of contamination, more camel milk analyzed does not originate from sick animals or carriers. The milk is not contaminated either by carriers or diseased individuals.

The rate of sulphite-reducing clostridia has a mean less than that described by the Algerian regulation (NA 35, 1998). However, their absence in 50% of the samples explains the absence of telluric contamination (Cuq, 2007). Due to the absence of a threshold for the enumeration of yeasts and molds, we compared with the microbiological limit values for camel milk from other work. Indeed, the data acquired for the enumeration of fungal flora in the samples analyzed are higher than that reported by Benkerroum *et al.,* (2003) (7.94 x10 fu/ml) and appears less contaminated compared to the given by El-Al-Ziney and Turki (2007) (10^7 fu/ml). This can be interpreted by the fact that the slightly acidic pH of camel milk is making predominate bacteria than fungal flora.

Conclusion

Camel milk is an essential food resources in arid, however its valuation remains very restricted until now. Although camel milk is subject to numerous works in the world in recent years, there is very little research that has carried on the camel milk produced in our region, in particular the assessment of its hygienic quality and the study of its physico-chemical and nutritional properties. Through this study, we tried to make a modest contribution to enrich the knowledge of milk which we have surrounded biochemical, physico-chemical and microbiological analysis of this product. Biochemical and physico-chemical analysis of samples showed that camel milk has a relatively high acidity 4.49 g/l, with a slightly acidic pH (5.67). The fat content and density are relatively low (29.87g/l and 1.0268 respectively), this is confirmed by the low levels of total dry extract (89 g/l). The relatively degraded hygienic quality appears affect the lactose content (28.18 g/l). The results of microbiological analysis suggest that most of the raw milk samples examined have a microbiological quality swung of satisfactory and acceptable that the presence of coagulase-positive staphylococci in some samples analyzed which present an unacceptable quality may pose a risk to consumer health.

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