

Assessment of Quality of Camel Milk and Gariss, North Kordofan State, Sudan

¹Adam Ismail Ahmed, ¹Abdelrahman Ahmed Mohammed, ²Bernard Faye, ³Lucie Blanchard and ⁴Sallam. A. Bakheit

¹Department of Biochemistry & Food Science, Faculty of Natural Resources & Environmental Studies, University of Kordofan, Elobeid, Sudan, P.O. Box .160.

²Centre De Coopération Internationale En Recherche Agronomique Pour Le Développement CIRAD, Montpellier, France.

³Ecole Nationale Vétérinaire De Nantes, Nantes, France.

⁴Department of animal production, Faculty of Natural Resources & Environmental Studies, University of Kordofan, Elobeid, Sudan, P.O. Box .160.

Abstract: The present study was conducted near Elobeid north Kordofan state, Sudan to assess the quality of camel milk and gariss. The total viable count of bacteria was measured accompanied by the enumeration of other flora on milk and gariss, then pathogenicity of the milk and gariss study by presence of coliform bacteria mainly *Escherichia coli*.

Key words: Camel milk, Gariss, *Escherichia coli*, total viable count of bacteria, microbial contamination of milk and fermentation Container.

INTRODUCTION

The camel (*Camelus dromedarius*) is of significant socio-economic importance in many arid and semi-arid parts of the world and its milk constitutes an important component of human diets in these regions. Milk is the main source of nutrition for the neonate calves, and provides all the essential nutrients for growth and development, e.g. proteins, minerals, carbohydrates, fatty acids, growth factors, immune modulators, etc. ^[11].

Milk is a good medium for several bacteria to develop. The growth of bacteria in milk depends mainly on temperature and the presence of other bacteria ^[13]. As camel milk is usually consumed in its raw state, the presence of pathogenic bacteria may be of public health importance besides its influence on animal health ^[14,10]. Generally, bacteria in milk can occur through colonization of the teat canal or an infected udder (clinical or subclinical mastitis), respectively, or as contaminants. Normally, the first contamination of milk takes place in the moment of milking during the passage of the teat canal and by milking equipment or milking personal. Further on contamination is possible during transport and storage of the milk. The main reasons for spoilage of milk are saprophytic microorganisms. Mastitis pathogens, as far as they are zoonotic, are of public health concern as some of them are capable of producing toxins or causing infections in man ^[13,12].

Therefore the objective of the present investigation to study the total viable count of bacteria, coliform and pH of raw and fermented camel milk procured from nomads' camps and market near Elobeid north Kordofan state, Sudan.

MATERIAL AND METHODS

Samples: Samples of raw milk were carried out in 5 farms situated near Elobeid at rate of 5 camels in milk per farm. The visits were not made on time trafficking, camels sampled were chosen based on the presence in their udders calf. Two samples of Gariss and milk have also been carried out on the market of Kerima.

In the farms, samples of milk were made by nomadic (shepherd) in charge of the herd and milking. If females wore Surar, it was removed to find themselves in conditions of trafficking. Plastic containers were sterile used. The Gariss was collected using a sterile plastic pipette and placed in a tube identical. On the market Kerima, samples were made directly in containers presented with a sterile pipette. Samples from the camps and markets were then transported under refrigeration (in a water-ice in a cooler) to the laboratory of microbiology, Department of biochemistry and food science, University of Kordofan. They were then placed in a refrigerator until completion of the different tests.

Methods:

Microbiological Analysis:

Enumeration of Mesophilic Aerobic Flora Total: To estimate and compare the standards of the Public Health Sudanese overall bacterial contamination of samples, enumeration of aerobic flora Total mesophilic was performed on each sample of raw milk and Gariss. The middle nonselective Plate Count Agar (PCA) Laboratory Merck ® was used. Dilutions successive samples were taken. For each sample, three Petri dishes were sown in depth with 1 ml of three successive dilutions. The Petri dishes were incubated at 30 ° C for 48 hours. A count colony was performed after 24 and 48 hours of incubation.

Estimation of Bacteria Other than Lactic Flora in Fermented Milk:

The selective medium Count Agar Sugar-free (SF) 4 Laboratory Merck ® was used. This medium is used for the enumeration of "contaminants" in butter and products milk. Devoid of fermentable sugar, low nutritional value, growth lactic acid bacteria is inhibited in favor of less demanding bacteria in nutrient development, bacteria designated as "contaminants" because outside a prior not specific to the flora of dairy product. The growth of bacterial strains on this medium indicates contamination and / or the possible survival of organisms in the fermented product. Of serial dilutions of samples of Gariss have been made and the Petri dishes were sown on the surface with 0.1 ml before being incubated at 35 ° C for 48 hours. A colony count was performed after 24 and 48 hours of incubation.

Estimated Population of Coliform:

MacConkey broth ^[15] is a culture medium used for selective detection of bacteria belonging to the family Enterobacteriaceae. The medium was divided into tubes each containing 10 ml of broth and Durham tube was overthrown in each. The set was sterilized by autoclaving (121 °C, 15 minutes). The method of most probable number three tubes was used. This test to estimate the most probable number of coliform potential in milk sampled. Three Successive decimal dilutions of each sample were prepared. For each dilution decimal, three tubes of MacConkey broth were each inoculated with 1 ml of corresponding solution before being incubated at 30 ° C for 48-72 hours. Reading tubes (to be 9 per sample) was performed after 24 and 48 hours of incubation. Content each positive tube must then be cultured in a new broth MacConkey heated to 44 ° C, and incubated in a water bath at the same temperature (44 ±0.25 ° C) for 24 hours, which is the Eijkman test. Gas production and acid production indicates the presence of Escherichia coli type 1 and to estimate the most probable number of these germs in the sample.

Measurement of pH:

Measurements of pH of each sample were performed using the pH meter: "PH HANNA Instruments pH 211 Microprocessor pH Meter" on the remaining samples after culturing for microbiological testing.

RESULTS AND DISCUSSION:

The sample G5 has the highest pH. The farmer is the only five that does not use of Gariss yesterday for the preparation of Gariss daily (table3). We can notice that for samples G4 and G5, the same type of container in plastic is used; the fermentation time is approximately the same as those total viable counts and other than lactic acid. However, the difference in pH sensitive, with almost 2.5 units apart. The sample G4 comes from the addition of raw milk Gariss from yesterday, onion, fenugreek and black cumin seeds. For the same enumeration of total flora, the proportion of lactic acid bacteria is probably higher for the sample G4 and the fermentation process more important in this in the sample G5. To this can be added the influence of the addition of condiments. The samples G2 and G4 are prepared using the previous Gariss and the same mixture of spices. The respective times are close of fermentation (6 and 7 hours) of same for their respective pH (3.86 and 3.51). The containers used are different, a further goatskin for livestock No. 2 and a jerry can for No. 4. Counts Flora Total and flora other than milk are higher for sample G2 (2.80×10^5 ufc.mL-1G2 cons to 5.80×10^4 ufc.mL-1 to G4 and 6.80×10^6 ufc.mL-1 for G2 and 8.35×10^3 ufc.mL-1 to G4). This can partly be explained by the macroscopic cleanliness of Gariss.

The G2 sample contained many twigs floating on its surface when we were presented in a bowl for sampling. The Gariss came directly from the addition, twigs were therefore present before in it and have played the role of vector microorganisms. A difference in the amount of added Gariss could influence enumeration of total viable but would not influence the flora other than lactic agar revealed on "Sugar Free". Indeed, despite a pH below 4, flora other than milk is high in the sample and G2 is almost twice that present in the sample G4. The assumption of exogenous contamination at the stage of raw milk by germs uncompetitive compared with lactic ferments is possible. The hypothesis of a poorly cleaned bowl in which we conducted the sampling is less likely, since the Gariss was already a highly acidic, unfavorable to development of newly introduced bacteria.

Regarding the samples G1 and G3 prepared with only the Gariss day, the fermentation time and the containers are different. Counts Flora Total in these

samples is higher respectively 1.79×10^6 and 2.70×10^6 ufc.ml-1. The macroscopic appearance of Gariss does not explain this difference other samples. However, a clear difference between these two samples Gariss since the G1 has been fermented for only 4 hours before sampling. This indicate either a significant contamination of raw milk to ferment (which our analysis the milk of five camels livestock does not appear to support) or a significant amount yeast retained for the preparation of Gariss. The pH also differ significantly (5.48 against 4.08, respectively). This can be directly related to the Unlike short fermentation time for sample G1 for less than the length of the lag phase for growth of bacteria in camel milk (7 hours according to El Zubeir and Marowa in 2009 and 5 hours for Attia et al.,^[9]). As concerning the flora other than lactic counts are high (1.55×10^4 and 5.20×10^5 ufc.mL-1) despite a low pH for sample G3 (see above). As G2 for breeding, it could be a contamination of milk with exogenous survival germs in Gariss. The Gariss appeared macroscopically clean. However, it is prepared and stored in plastic bottles reused. Improper cleaning or cleaning done with water of poor microbiological quality can be origin. For Gariss G1, the pH is still relatively high after only 4 hours fermentation, it is difficult to interpret compared with the other samples Enumeration of lactic flora not because germs are able to survive and multiply in these conditions.

Comparing G3 with G2 and G4, the pH of the latter are much lower than G3 (pH 2 = 3.86, pH 4 = 3.51; pH3 = 4.08) which has a fermentation time higher. All are prepared with the help of Gariss. The difference in pH could then find its home again in the quantity of starter used, or the rapid decline of pH G2 and G4 would be correlated with the addition of spices.

Quality Overall microbiological milk and Gariss:
Enumeration of the microbial flora of raw milk.:
The overall low microbial contamination of raw milk is consistent with the standards Sudanese food security. These standards do not specify, however, if the camel milk must meet these same criteria previously established for milk from other animals, including cows. In France, milk quality imposed on farmers for sale of milk is a count of the microbial flora of less than 5×10^4 cfu/ml^[16]. All samples of camel milk collected during this study would meet this criterion. However, these results do not consider the contamination from milking equipment because the samples were collected directly at the worst camels. In literature, the microbial counts of total higher are identified. In Mauritania, Tourette and his colleagues have demonstrated a FAMT average of 1.65×10^6 cfu/ml [0 - 3.46×10^6] after taking in the bowl of

treats^[6]. Nevertheless, our results are consistent with those found in Sudan by Mirghany^[1], which showed a mean total flora in raw milk 4.2×10^2 cfu/ml, and on a larger scale, those Wernery *et al.*,^[7], which tested 313 samples of milk. Nearly 95% (94.90%) of them met the main criteria of quality in terms of food security in force in Europe in For 67 samples did not meet the requirements in Europe, 42 (62.69 %) Had a total bacterial flora than the threshold of 50 000 germes.mL-1, 19 (28.36%) did not meet the requirements on the Coliform and 6 (8.96%) were not meet the criterion on *Staphylococcus aureus*. To explain these variations between studies, the season could be taken into account. Indeed, many farmers reported episodes of diarrhea, on the whole herd during the ingestion of certain plants in the pasture and fresh herbs in season rains. The chemical composition of plants at the beginning of the rainy season could be to cause some diarrhea herd this season. These episodes may contribute to contamination of the hind limbs and affect the cleanliness of the udder. Of moreover, during the rainy season, camels lie in areas more or less muddy and have soiled udders. Higher bacterial counts have been detected in milk collected from farmers for good tech over those of milk collected from farmers taking fewer precautions. The main factor was affecting the cleanliness of the udder^[6]. Breeders encountered clean breast of the back of the hand to get rid of the dust sand or do not clean. This process may be inadequate rains and facilitate the microbial contamination of milk.

During migration there is the problem of water availability. Few farmers have discussed this problem but it seems that access to water is difficult and that it not be available for cleaning milking equipment. The transport of water for a wet cleaning of the udder before milking seems very difficult to look for pastoralists did not gain water sources once per month or every two months. Not forgetting the important role of the quality of water used.

The lesions observed in the breast are numerous and mainly due to the posed by the keeper with an anti-feeding. They are described in the bibliography.^[4,5] However, in our study the conformation of the teat does not appear to influence the contamination of milk by mesophilic aerobic bacterial flora. This may be due to small numbers studied, at the age of lesions and season. Indeed, many deformations appeared old, healed or healing. In addition, the breasts are unique to this time of year perhaps the role of injury time that pathway of germs in the breast still less. However, lesions of the teats are a recognized risk factor^[3] in the onset of mastitis.

Table 1: Results of pH measurements of samples of milk.

Sample/livestock	1	2	3	4	5	Mean
A	6.43	6.58	6.68	6.29	6.56	6.51±0.151
B	6.59	6.58	6.23	6.48	6.42	6.46±0.147
C	6.24	6.72	6.71	6.49	6.51	6.53±0.197
D	6.45	6.47	6.27	6.71	6.39	6.46±0.161
E	7.06	6.28	6.72	6.93	6.33	6.66±350

- Each value is an average of three experimental samples analyzed in triplicate.
 - Values are means ± (standard deviation).
 - Means in a column are significantly not different.

Table 2: Results of counts of total viable and lactic other than in samples of Gariss:

Gariss sample	APC	Enumeration "Other flora" than lactic bacteria	Fermentation time (hours actual/ theoretical)	pH	fermentation Container
G1	1.55×10 ⁴	1.79×10 ⁶	4/5	(5.48) ^f ±0.12	Siin
G2	6.80×10 ⁶	2.80×10 ⁵	6/7	(3.86) ^b ±0.14	Siin
G3	5.20×10 ⁵	2.70×10 ⁶	12/12	(4.08) ^d ±0.08	Plastic bottle
G4	8.35×10 ³	5.80×10 ⁴	7/3 ^a	(3.51) ^e ±0.14	Baga plastic
G5	7.00×10 ³	2.30×10 ⁴	8/24	(5.99) ^a ±0.09	Baga plastic

^aThe Gariss can be drunk from 2 to 3 hours of fermentation.
 - Each pH value is an average of three experimental samples analyzed in triplicate.
 - pH values are means ± (standard deviation).
 - pH means in a column sharing a common superscript letter are significantly not different.

Table 3: Summary of criteria for preparation of Gariss livestock farmers of the five farms sampled.

Sample	Starter	Added ingredients
G1	Gariss	Nothing
G2	Gariss	Onion, funogreek and Black Cumin Seeds
G3	Gariss	Nothing
G4	Gariss	Onion, funogreek and Black Cumin Seeds
G5	no gariss	Nothing

Comparison of samples of Gariss and enumeration of flora: Lactic the high pH of the G5 prepared unleavened coincides with the explanation collected from Women on the market Kerima recommending drinking this type of Gariss only day. The preparation of Gariss unleavened would be longer, probably due to Competition largest lactic acid bacteria with other floras contained in the raw milk. When contamination of raw milk, various contaminants cannot be eliminated during fermentation. They can survive with limited growth or inhibited, or as a spore. Some of these germs could develop resistance to acidity Tsegaye Ashenafi ^[2]. It would be interesting to evaluate the influence of adding different seasonings in quantities on the pH of Gariss. Mirghany ^[1] did not find at 5% for onions and 1% of density for black cumin seeds, but one suspects significant role of this practice on fermentation. The questioning of farmers on quantities necessary to obtain a Gariss is a first step before

studying their influence on the pH and possibly microbial contamination.

We can assume the existence of a link between some of them and encourage the study of these parameters to know the implications for the quality of Gariss. Thus, it would be interesting to explore, the influence of the quantity of starter used in the kinetics of the pH of milk used to ferment Gariss, and consider whether there is a link with the survival of organisms other than lactic acid in the finished product; The link between the quantity of starter used and the fermentation time needed obtaining the Gariss? the influence of the addition of spices on the kinetics of the pH of fermented milk and of Gariss this time taking into account the quantity and frequency with which farmers add or change the spices; The influence of the addition of spices on the microbiological characteristics of finished product? The influence of using a cloth to hold the spices added on the

microbiological quality of fermented milk? Influence the type of container (siin and others) on these parameters.

ACKNOWLEDGMENT

We duly acknowledge the financial support received from French Embassy Khartoum also Staff and colleagues from Faculty of Natural Resource and Environmental Studies Dr.Abdalla.M .Abdalla, Mr. Wail M Haroun, Mr. Omer Abdelhadi and Mr. Asadig ABdelbasit.

REFERENCES

1. Mirghany, A.A., 1994. Microbiological and biochemical properties of the fermented camel milk Gariss. M.Sc. thesis, Faculty of agriculture, University of Khartoum, Sudan.
2. Tsegaye, M. and M. Ashenafi, 2005. Fate of *Escherichia coli* O157:H7 during the processing and storage of Ergo and Ayib, traditional Ethiopian dairy products. International Journal of food microbiology, 103: 11-21.
3. Younan, M., 2004. Milk hygiene and udder health. In: FARAH, Z and FISCHER A. Milk and Meat from the camel: handbook on products and processing. Zurich. Eds Farah and Fischer., 232: 67-76.
4. Obieda, A.I. and H.O. Bagadi, 1996. Mastitis in *Camelus dromedarius* and the somatic cell content of camel's milk. Research in Veterinary Science., 61: 55-58.
5. Abdurahman, O.A.S., H. Agab, B. Abbas and G. Aström, 1995. Relations between udder infection and somatic cells in camel (*Camelus dromedarius*) milk. Acta Veterinaria Scandinavica., 36(4): 423-431.
6. Tourette, I., S. Messad and B. Faye, 2003. Interactions entre les pratiques de traite et la qualité sanitaire du lait de chamelle en Mauritanie. In : *Lait de chamelle pour l'Afrique*. FAO Production et santé animales, Rome, 2004. Atelier sur la filière laitière caméline en Afrique. Niamey, 5 - 8 Novembre, 2003, 222: 61-70.
7. Wernery, U., H. Becker and E. Maertlbauer, 2002. Microbiological status of raw dromedary milk. Journal of Camel Practice and Research., 9(1): 1-4.
8. Elzubeir, I.E.M. and I. Marowa, 2009. Effect of pasteurization of milk on the keeping quality of fermented camel milk (*Gariss*) in Sudan. *Livestock Research for Rural Development*. 2009, 21, Article No19. Retrieved August 11, 2009, from <http://www.lrrd.org/lrrd21/2/zube21019.htm>.
9. Attia, H., N. Kherouatou and A. Dhoub, 2001. Dromedary milk lactic acid fermentation: microbiological and rheological characteristics. Journal of Industrial microbiology and Biotechnology., 26(5): 263-270.
10. Farah, Z. and A. Fischer, 2004. Milk and Meat from the Camel: Handbook on products and processing, FAO, Rome, Italy (www.fao.org.)
11. Elhatmi, H., J.M. Girardet, J.L. Gaillard, M.H. Yahyaoui and H. Attia, 2007) Characterization of whey proteins of camel (*Camelus dromedarius*) milk and colostrums, Small Ruminant Research, 70 : 267-271.
12. Semereab, T., B. Molla, 2001. Bacteriological quality of raw milk of camel (*Camelus dromedarius*) in Afar region (Ethiopia). J. Camel Pract. Res., 8: 51- 54.
13. Heesch, W., 1994. Milch als Lebensmittel. In: Wendt, K., H. Bostedt, H. Mielke & H.-W. Fuchs (Eds): Euter- und Gesäugekrankheiten. Gustav Fischer Verlag, Jena, Stuttgart, Germany, pp: 138 - 180 cited in Eberlein, 2007.
14. Saad, N.M. and A.E.R. Thabet, 1993. Bacteriological quality of camel's milk with special reference to mastitis, Assiut Vet. Med. J., 28: 194 - 199.
15. Harrigan, W.F. and M.E. McCance, 1966. Laboratory Methods in Microbiology. New York, pp: 362.
16. MAGRAS, C., E. DROMIGNY et F. TARTROU, 2007. La filière lait et produits laitiers - éléments et facteurs de qualité. *Polycopié d'enseignement ENVN.*, UV 82(4): 74.