MEDWIN PUBLISHERS
Committed to create value for Researchers

Food Science and Nutrition Technology
ISSN: 2574-2701

Microbial Loads of Gariss Collected During Movement and Settlement of Nomadic Camel Herders in Algadarif State, Sudan

Research Article
Volume 1 Issue 2
Received Date: July 21, 2016
Published Date: August 25, 2016

Suliman ESK and El Zubeir IEM*

Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Sudan

***Corresponding author:** El Zubeir IEM, Department of Dairy Production, Faculty of Animal Production, University of Khartoum, PO Box 321, Khartoum, Sudan, Tel: +249 185 321246; E-mail: Ibtisammohamed@hotmail.com

Abstract

This study was conducted in AlGadarif State to assess the microbial quality of Gariss; prepared by nomadic women camel herders and to evaluate the effects of different types of containers and additives used on the microbial loads. The samples (n= 19) collected during settlement and movement of herders were significantly (P<0.05) higher in total bacterial count (TBC) (7.32 ± 0.03) and lower in *Lactobacillus spp.* Count (7.06 ± 0.03) and *Streptococcus spp.* count (7.13 ± 0.29) compared to that collected during settlement. Gariss prepared in bukhsa was significantly (P<0.05) higher in *Lactobacillus spp.* count and it showed the lowest mean for TBC. Gariss samples prepared in plastic containers were significantly (P<0.05) lower in coliform bacterial count and higher in TBC. The lowest counts of *Lactobacillus* and *Streptococcus spp*. (P<0.05) were found in Gariss prepared in stainless steel containers. The highest TBC, *Lactobacillus spp.* count and yeast count were observed in Gariss samples prepared using no additives. The additives used were significantly (P<0.05) increased the number of *Streptococcus spp*. as they enhance fermentation. The isolates identified were *L. fermentum* (26.67%), *L. brevis* (13.33%), *L. plantarum* (20%), *L. acidophilus* (20%), *L. casei* (13.33%), *L. delbrueckii* (6.67%), *St. thermophilus* (80%) and *St. lactis* (20%). Moreover the coliform bacteria were found as *E. coli* (40%), *Klebsiella* (30%), and the *Enterobacter aerogenes* (30%). The present study concluded that the microbial loads were affected by movement seasons, types of additives and containers used. It recommended to control the fermentation by using improved starter culture and more studies are needed on the effect of the additives and containers on traditional Gariss quality.

Keywords: Nomadic women; Camel milk fermentation; Gariss processing, containers; Added spices; Microbial quality; Sudan

Introduction

A variety of foods can be preserved by lactic acid fermentation, which is the most widely used acidification process to coagulate milk during the manufacture of cultured dairy products [1]. The fermented products of camel's milk have shown that they have unique and different microflora depending on the production

technology as well as on the ecological localities where they have been produced [2-4]. Traditionally, fermented camel milk is allowed to ferment naturally without prior heat treatment and without addition of starter cultures [2,5,6]. The method of Gariss preparation was described [2-5,7-9]. Gariss is a special kind of full cream fermented camel milk in Sudan. It is made by a semi-continuous or fed-batch fermentation, whenever fresh camel milk is added to the Siin part of the fermented product has been consumed and it's widely consumed by the pastoralist communities living in the arid and semi-arid regions of the country [4,5,9]. Gariss differs from other kinds of Sudanese fermented camel milks in that it has substantial amounts of ethanol [5].

Several reports clearly show the difficulty of producing fermented camel milk products with high consistency due to the problem associated with milk coagulation [3,4,7-12]. On the other hands, evaluation of the LAB (Lactic acid bacteria) isolated from Gariss samples revealed variations in the predominance of *Lactobacillus spp.* in traditionally fermented camel´s milk [4,7,13,14]. Camels are playing very important contribution in the social and economic life of nomadic tribes as it is major source of food [15-17]. In Sudan, nomadic women usually processed camel milk into Gariss (fermented product) with variable flavors [9]. However some sporadic cases of toxicity were reported after consumption of Gariss. The objectives of this study are to assess the microbiological quality of Gariss prepared by nomadic camel women herders in Butana area (AlGedarif State) and to isolate and identify the predominant microflora in Gariss.

Materials and Methods

Area of study and target groups

AlGedarif State, which is located in the eastern of Sudan, is the selected area to perform this study. The nomadic women camel herders chosen for this study belong to the Lahaween tribes who stay in Butana plains in the northern part of Al Gedarif State during the rainy season (May to October) and then moved towards the southern part of the state from November-April to take maximum advantage of the natural grazing and water sources. Nomadic livestock owners who used to find ample dry season resources (water + grazing) in the Atbra valley now traverse the area and take their animals across the border with Ethiopia. In most cases in the dry season nomads, buy the crop residues remains from irrigated schemes after the harvest.

Collection of samples

Samples of Gariss were collected from two localities that include Fashga and Alsobag (part of Butane area located in AlGedarif State). The nomad's housekeepers usually processed Gariss using different types of containers and additives. The containers used include siin (Plate 1), bukhsa (Plate 2), plastic and stainless steel containers. Each Gariss sample was collected into sterile McCarteny bottles (10 ml). The samples were collected over a period of 1-2 days and kept at 4°C until being brought to Khartoum in an ice bag. The pH of each sample was measured at field and the microbiological analyses were performed in the Department of Dairy Production, University of Khartoum.

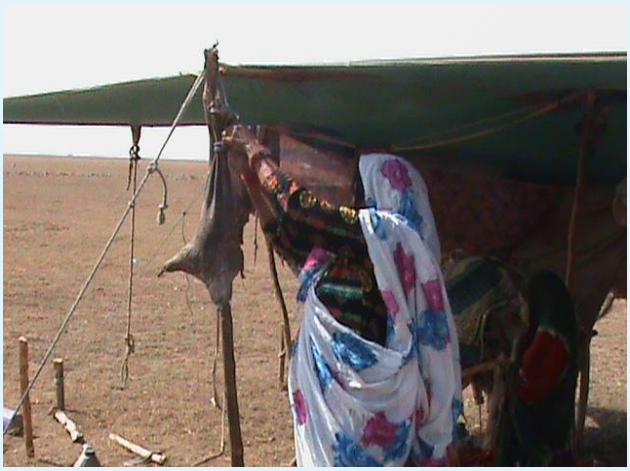

Plate 1: A nomadic women trying to hang her Siin containing Gariss.

Microbiological examinations of Gariss samples

The samples were collected into clean sterile bottles and transported in ice bag; the analysis was done within 48 hours. The collected samples were tested for standard plate count, coliform count, *Lactobacillus spp.* count, *Streptococcus spp.* count, and yeast count. Moreover, identification was done on the isolates from coliform, *Lactobacillus spp.* and *Streptococcus spp.*

All the media were obtained in dehydrated form and prepared according to manufacturer's instructions. Plate count agar medium (Hi Media M091) was used for total bacterial count, Eosin methylene blue agar (EMB) (Conda Cat no. 1039) was used for isolation of Enterobacteriacae, MacConkeys agar (Hi Media M 081) was used for detection and enumeration of coliform bacteria and Potato dextrose agar (Hi Media M 096A) was used for

enumeration of yeasts and moulds [18]. MRS broth (Hi Media M369 modified 1000 ml) was used for enumeration and isolation of *Lactobacillus spp.* and M 17 broth (Hi Media M929) was used for isolation of *Streptococcus spp.* [18].

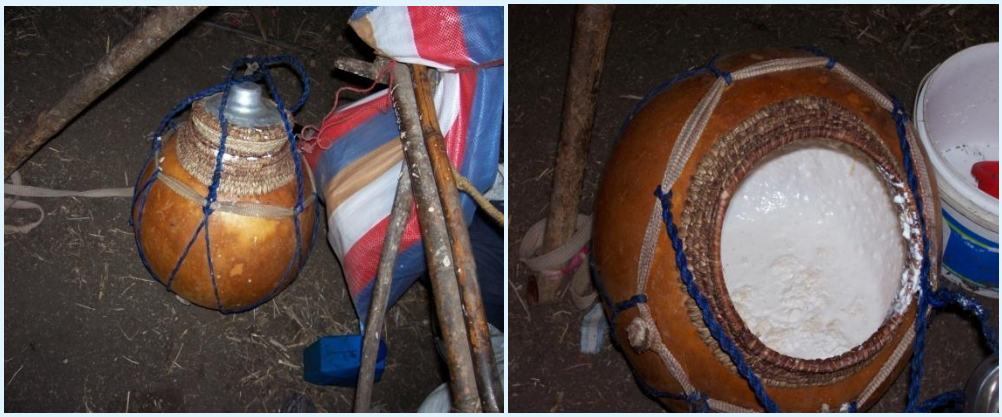

Plate 2: Bukhsa which is used to preserve Gariss.

Sterilization of equipments and media were done according to Barrow and Feltham [19]. The preparation of samples dilutions were done as described by Harrigan and McCance [18]. Plating, incubation and counting were done according to the methods outlined by Marshall [20]. Isolation, purification and identification (based on morphological, biochemical and sugars fermentation tests) were also done using the standard procedures [18,19]. The primary tests used include Gram's stain, catalase test, oxidation fermentation test, glucose test, motility test, the ability to grow at 15°C and 45°C. The confirmatory secondary tests used to confirm the isolated bacteria include sugars fermentation (rafffinose, arabinose, lactose, salicin, melibiose, melezitose, maltose and glucose), methyl red test, Vogues Proskauer test, indole test, urease test and citrate utilization.

Statistical analysis

The data were analyzed using completely randomized design. The significant differences between means were determined using the Least Significant Different (Statistix 8).

Results and Discussion

The Gariss processing practiced by the nomadic women camel herders in AlGadarif State showed that different types of bacteria, which affect the microbial quality of Gariss (Tables 1 and 2). The results in Table 1 showed that the mean log of total bacterial count (TBC) in the Gariss collected during movement of nomadic camel herders (7.32±0.03) was significantly ($P<0.05$) higher compared to that collected during settlement (6.92±0.06). This might be because during the rainy season (movement season) a lot of sandy winds are common and the containers were used continuously without renewable or cleaning. The mean log of TBC in Gariss was found previously to range between 7.3 and 8.7 cfu/ ml [4,13]. Whereas, the means log of *Lactobacillus spp.* count (7.06 ± 0.30) and *Streptococcus spp.* count (7.13±0.29) were significantly ($P<0.001$) higher in the Gariss collected during settlement compared to that estimated during movement season (Table 1). This might be due to the continuous addition of raw fresh camel milk during movement season as camels are grazing far in the pasture. The range of the log *Streptococcus spp*. and *Lactobacilli spp*. counts were 7.3–8.4 and 7.8–8.7 cfu/ml, respectively [13]. However lower log of *Streptococcus spp*. count (6.5–6.9) and *Lactobacilli spp.* count (6.6– 6.8), respectively were reported previously [4].

There was no significant ($P>0.05$) differences between the Gariss collected during settlement or during movement of nomadic camel herders for the log yeast count (7.03±0.23 and 6.92±0.25, respectively) and the log coliform count (7.28±0.17 and 7.17±0.26, respectively) as shown in Table 1. The range of yeast log count in Gariss samples revealed 6.05–8.42 cfu/ml [4,13,21,22]. The variations might also be due to the differences in the methods of preparation of fermented camel's milk [2,3,5,9]. The differences could be attributed also to the

variations in temperature between the two seasons. Variations were found on the microbial loads for Gariss samples that kept at 37°C and 25°C [23]. Moreover the nature of fermented products is different from one region to another and this depends on the local indigenous microflora, which in turn reflects the climatic conditions of the area [24].

Parameters	Movements				Settlement			SE
(Log CFU/ml)	Means	Max	Min	SE	Means ± S. d	Max	Min	
TBC	7.32[a]	7.51	6.95	0.02	6.92[b] ± 0.06	6.99	6.81	0.05
Streptococcus spp	7.13[a]	7.52	6.54	0.1	7.41[b] ± 0.21	7.52	6.91	0.1
Lactobacillus spp	7.06[a]	7.52	6.51	0.03	7.45[b] ± 0.08	7.53	7.32	0.09
Yeast	7.03[a]	7.42	6.53	0.03	6.92[a] ± 0.25	7.3	6.6	0.08
Coliform	7.28[a]	7.51	6.92	0.02	7.17[a] ± 0.26	7.5	6.89	0.07

Table 1: Microbial loads of Gariss samples collected during movement and settlement of nomadic camel herders, AlGadarif State

[a , b]: Mean values within the same row with different superscripts letters are significantly different at P<0.05.
SE: Standard error of means.

The results shown in Table 2 indicated that the types of additives used in Gariss preparation revealed significant (P<0.001) variations on mean log counts of microorganisms investigated. The highest mean log of total bacterial count (7.40±0.05) was observed in Gariss samples prepared using no additives (7.40±0.05). Whereas, the lowest means log of total bacterial count were found in Gariss samples prepared using additives mixtures. This could be due to the fact that most of the additives used were proved to have antimicrobial properties. The mean log count of coliform bacteria in the Gariss samples prepared using ginger and black cumin (6.91±0.09) was significantly (P<0.05) lower when compared to Gariss either prepared using no additives (7.27±0.05) or prepared using the other additives mixtures. Similarly the highest (P<0.05) count of yeast was observed in Gariss samples prepared using no additives (7.22±0.04). This result supported some of the previous findings [3,6]. Moreover, the results obviously showed that using additives for preparing Gariss significantly (P<0.05) increased the number of *Streptococcus spp*; one of the major fermentative organism in Gariss; probably by eliminating some of the contaminant present in the products. This goes in line with the previous recommendation that the processing of Sudanese fermented milk (*mish*), using spices like black cumin, fenugreek, garlic and other known spices, since those spices were proved to have significant effect as preservative [25].

Additives (species)	Coliform (log cfu/ml)	TBC (log cfu/ml)	*Lactobacillus* (log cfu/ml)	*Streptococcus* (log cfu/ml)	Yeast (log cfu/ml)
Blank Cumin	7.27[a]±0.05	7.40[a] ±0.05	6.94[abc]±0.07	6.97[b] ±0.07	7.22[a] ±0.04
Ginger+ Cumin	6.91[b]±0.09	6.94[c]±0.12	7.51[a]±0.17	7.36[a]±0.18	6.63[d]±0.10
Onion+ Fenugreek	7.21[a]± 0.06	7.20[b]±0.05	7.05[abc]±0.08	7.16[ab]±0.08	6.80[cd]±0.05
Cumin + Onion	7.35[a]± 0.09	7.38[ab]±0.08	7.09[a]±0.12	7.17[ab]±0.13	6.95[bc]±0.07
Ginger +Cumin +Onion	7.41[a]± 0.09	6.84[c]±0.08	7.48[ab]±0.12	7.51[a]±0.13	7.10[ab]±0.07
Cum+Fenugreek +Grangal+ onion	7.26[a]± 0.07	7.30[ab]±0.07	7.40[a]±0.10	7.39[a]±0.10	7.03[b]±0.06

Table 2: Variations of the log of the microbial counts as affected by different species added during the processing.
a, b, c, d: Mean values within the same column with different superscripts letters are significantly different at P<0.05.

The highest means log of total bacterial count (7.31±0.04) estimated for Gariss samples prepared in plastic and siin containers (7.30±0.04). However, the lowest mean log (6.94±0.08) was observed in Gariss samples prepared in bukhsa container. Whereas, the log means counts of *Lactobacillus spp* (7.48±0.13) and *Streptococcus spp*. (7.36±0.13) in Gariss samples prepared in bukhsa were significantly (P<0.001) higher compared

to Gariss kept in other containers. The highest counts of *Lactobacillus spp.* and *Streptococcus spp*. of the Gariss samples made in bukhsa could be related to the suitable environment as its material (woody) and the pore help to preserve the starter culture activities more than other materials. The lowest mean log count of *Lactobacillus spp.* (6.54±0.18) and *Streptococcus spp.* (6.54±0.19) were found in Gariss samples prepared in stainless steel containers (Table 3). This could be attributed to the reason that stainless container absorbed the heat more compared to the other used containers. However the results in Table 3 reflected that the types of containers had no significant (P<0.05) effect on log yeast count. The mean log count of coliform bacteria in Gariss prepared in plastic containers (7.14±0.04) was significantly (P<0.001) lower compared to that of Gariss prepared in bukhsa (7.40±0.09), siin (7.32±0.04), and stainless steel containers (7.49±0.12). No growth of coliform bacteria in Gariss was observed before [4]. However the range for the log of coliform present in Gariss was 3.2–3.5 cfu/ ml [22]. The data showed the highest coliform count in Gariss samples was found in plastic containers, which reused continuously and it is not changed [3]. Moreover when the product is prepared and stored in the plastic containers without cleaning or the improper cleaning because of the difficulty to reach the bottom and/or the cleaning is done with water of poor microbiological quality. The presence of coliform in milk and milk products is an indication of unsanitary production and or improper handling of either milk or milk utensils [26].

Types of Containers	Coliform (log cfu/ml)	TBC (log cfu/ml)	*Lactobacillus spp.* (log cfu/ml)	*Streptococcus spp.* (log cfu/ml)	Yeast (log cfu/ml)
Bukhsa	7.40^{a}± 0.09	6.94^{b}± 0.08	7.48^{a}± 0.13	7.36^{a} ±0.13	7.10^{a} ±0.12
Plastic	7.14^{b}± 0.04	7.31^{a}± 0.04	7.12^{b}± 0.07	7.20^{a} ±0.06	7.02^{a} ±0.06
Siin	7.32^{a}± 0.04	7.30^{a}± 0.04	7.17^{b}± 0.07	7.19^{a} ±0.06	6.95^{a} ±0.06
Stainless steel	7.49^{a}± 0.12	7.01^{ab}± 0.12	6.54^{c}± 0.19	6.54^{b} ±0.18	7.21^{a} ±0.16

Table 3: Variations of the log of the microbial counts as affected by different containers used for processing Gariss.
$^{a, b, c, d}$: Mean values within the same column with different superscripts letters are significantly different at P<0.05.

The identification of *Lactobacillus spp*. from Gariss samples revealed the presence of *L. fermentum* (26.67%), *L. brevis* (13.33%), *L. plantarum* (20%), *L. acidophilus* (20%), *L. casei* (13.33%), and *L. delbrueckii* (6.67%) as shown in Table 4. The *L. fermentum* is the most predominant bacterial species and it was found previously to be frequently isolated from Gariss [2,4,7,13,14]. The presence of *L. plantarum* in Gariss samples might be due to the reason that low rate of *Lactobacillus plantarum* is known to be commonly associated with plants. Thus in studies on the occurrence of lactic acid bacteria *Lactobacillus plantarum* constituted the highest number of *Lactobacillus spp*. isolated from fermented plant materials [6]. The previous study showed *Lactobacillus planturum*, *Streptococcus lactis* and *S. lactis* subsp *diactylactis* were found in high population compared to other bacterial isolates [4]. In contrast to Abdelgadir et al [13], *L. fermentum* and, *S. infantarius* subsp. *Infantarius* have dominant population. Similarly *L. plantarum* and *L. raffinolactis* were isolated as dominate (50%) of total bacteria isolated [7]. The dominance of mesophilic bacteria may be explained by the fact that the samples were collected in the cooler months and the ambient temperatures at which the natural fermentations of the tested samples took place favored proliferation of mesophilic bacteria [24].

The predominant *Streptococcus spp.* in Garisss samples (Table 4) was *St. thermophilus* (80%) and this goes in line with Hassan et al [4]. The coliform bacteria isolated from Gariss samples (Table 4) showed that *E.coli* represented the highest proportion (40%) followed by *Klebsiella* (30%) and the *Enterobacter aerogenes* (30%). The *E. coli* has been isolated from fresh camel milk [27]. The coliforms are associated with poor hygiene and their occurrence in the product may indicate a potential health risk [27]. Highly significant (P<0.001) differences in the microbial counts between raw and heat-treated camel milk were reported [28]. Moreover they found that the shelf life of heat-treated camel milk was high compared with raw milk samples. The traditional fermented milk products are usually primitive, compared to modern ways of food preparation [6]. The spontaneous fermentation of

unheated milk takes advantage of natural microflora inherent in milk and environmental contaminants [29].

No	Identified strain	No of isolates	Frequency (%)
Lactobacillus spp			
1	*L. fermentum*	4	26.67
2	*L. brevis*	2	13.33
3	*L. acidophilus*	3	20
4	*L. plantarum*	3	20
5	*L. casei*	2	13.33
6	*L. delbrueckii*	1	6.67
Streptococcus spp			
7	*St. thermophilus*	8	80
8	*St. lactis*	2	20
Coliform			
9	*E.coli*	4	40
10	*Klebsiella*	3	30
11	*Enterobacter aerogenes*	3	30

Table 4: The bacteria isolates from Gariss samples collected from nomadic camel herders, AlGadarif State.

Conclusion

It is concluded that camel milk fermentation is spontaneous using undefined bacteria at the ambient temperature. The microbial contents were affected by the seasons of the nomadic camel milk herders (settlement and movement), types of additives and containers used in the processing of Gariss. Hence it is recommended to improve the spontaneous traditional fermentation. Controlled fermentation using mesophilic lactic acid bacteria starter culture is a very important strategy to be developed and introduced. Further research is needed to characterize the properties of the isolated strains of the dominants lactic acid bacteria and yeasts.

References

1. Attia H, Kerouatou N, Dhouib A (2001) Dromedary milk lactic acid fermentation: Microbiological and rheological characteristics. J Ind Microbiol & Biotech 26(5): 263-270

2. Abdel Gadir WS, Ahmed TK, Dirar HA (1998) The traditional fermented milk products of the Sudan. Inter J Food Microbiol 44(1-2): 1-13

3. Ahmed AI, Mohammed AA, Faye B, Blanchard B, Bakheit SA (2010) Assessment of quality of camel milk and Gariss, North Kordofan State, Sudan. Research Journal of Animal and Veterinary Sciences 5(1): 18-22.

4. Hassan RA, El Zubeir IEM, Babiker SA (2008) Chemical and microbial measurements of fermented camel milk "Garris" from transhumance and nomadic herds in Sudan. Aust J Basic Appl Sci 2(4): 800-804.

5. Dirar HA (1993) Gariss. Dairy Products. The indigenous fermented foods of the Sudan. A Study in African Food and Nutrition. First edition, university press, Cambridge, UNIDO.

6. Shori AB (2012) Comparative study of chemical composition, isolation and identification of micro-flora in traditional fermented camel milk products: Gariss, Suusac, and Shubat. J Saudi Soci Agric Sci 11(2): 79-88.

7. Ashmaig A, Hasan A, El Gaali E (2009) Identification of lactic acid bacteria isolated from traditional Sudanese fermented camel's milk (Gariss). Afric Jl Microbiol Res 3(8): 451-457.

8. El Zubeir IEM, Ibrahium MI (2009) Effect of pasteurization of milk on the keeping quality of fermented camel milk (*Garriss*) in Sudan. Livestock Research for Rural Development 21(2).

9. Suliman ESK, El Zubeir IEM (2014) A Survey of the Processing and Chemical Composition of Gariss produced by nomadic camel women herders in Al Gaderif State, Sudan. Jord J Biol Sci 7(2): 95-100.

10. El Zubeir IEM, Jabreel MSO (2008) Fresh cheese from camel milk coagulated with Camifloc. Inter J Dairy Tech 61(1): 90-95.

11. Ahmed NAA, El Zubeir IEM (2011) Effect of salt level on some physical and chemical properties and acceptability of camel milk cheese, J Camelid Sci 4: 40-48.

12. El Zubeir IEM, Basher MAE, Alameen MH, Mohammed MA S, Shuiep ES (2012) Chemical characteristics and acceptability of yoghurt made from non bovine milks, Livestock Research for Rural Development 24(3).

13. Abdelgadir SW, Nielsen D, Hamad HS, Jakobsen M (2008) A traditional Sudanese fermented camel's milk product, Gariss, as a habitat of *Streptococcus infantarius* subsp. *infantarius*. Inter J Food Microbiol 127(3): 215-219.

14. Sulieman AE, Ilayan AA, El Faki AE (2006) Chemical and microbiological quality of Gariss, Sudanese fermented camel's milk product. Inter J Food Sci Techn 41(3): 321-328.

15. Farah Z, Mollet M, Younan M, Dahir R (2007) Camel dairy in Somalia: Limiting factors and development potential. Livestock Sci 110(1-2): 187-191.

16. Musa HH, Shuiep ES, El Zubier IEM (2006) Camel husbandry among pastoralists in Darfur in Western Sudan. Nomadic People 10(1): 101-105

17. Shuiep ES, El Zubeir IEM, Yousif IA (2014) Socioeconomic aspects of rearing camels under two production systems in Sudan. Livestock Research for Rural Development 26(11).

18. Harrigan, WF, MacCance ME (1976) Laboratory Methods in Food and Dairy Microbiology. Academic Press, London. pp 25-29.

19. Barrow GI, Feltham RKA (1993) Cowan and Steel's Manual for the Identification of Medical Bacteria. (Third edition), Cambridge University Press.

20. Marshall RT (1992) Standard Methods for the Examination of Dairy Products, 16th (ed), Washington, DC: American Public Health Association.

21. Hassan RA, El Zubeir IEM, Babiker SA (2007) Effect of pasteurization of raw camel milk and storage temperature on the chemical composition of fermented camel milk Inter J Dairy Sci 2(2): 166-171.

22. Sulieman AE, Osawa R, Tsenkova R (2007) Isolation and identification of lactobacilli from Gariss, a Sudanese fermented camel's milk product. Res J Microbiol 2(2): 125-132.

23. Hassan RA, El Zubeir IEM, Babiker SA (2006) Microbiology of camel fermented milk (Gariss) in Sudan. Res J Microbiolgy 1(2): 160-165.

24. Savadogo A, Ouattara CA, Savadogo PW, Baro N, Ouattara S, et al. (2004) Microorganisms involved in Fulani fermented milk in Burkina Faso. Pak J Nutr 3(2): 134-139.

25. Zubeir IEM, Abdalla WM, El Owni OAO (2005) Chemical composition of fermented milk (roub and mish) in Sudan. J Food Cont 16(7): 633-637.

26. El Zubeir IEM, Ahmed MI (2007) The hygienic quality of raw milk produced by some dairy farms in Khartoum-Sudan. Res J Microbiol 2(12): 988-991.

27. Shuiep ES, El Zubeir IEM, El Owni OAO, Musa HH (2007) Assessment of hygienic quality of camel (*Camelus dromedarius*) milk in Khartoum State, Sudan. Bull Anim Hlth Prod Afr 55: 112-117.

28. Mohamed IMA, El Zubeir IEM (2014) Effect of heat treatment on the keeping quality of camel milk. Ann Food Sci Tech 15(2): 239-245.

29. Zamfir M, Vancanneyt M, Makras L, Vaningelgem F, Lefebvre K, et al. (2006) Biodiversity of lactic acid bacteria in Romanian dairy products. Syst Appl Microbiol 29(6): 487-495.